

Summit times were here again

Last week's gathering of the seven major industrial nations of the world in Paris has produced a well-sounding communique, but no sense that the problems it describes will be quickly resolved.

THE conjunction last weekend of the summit meeting of the chief executives of the seven richest countries and the 201st anniversary of Bastille Day (cardinal and ordinal numbers are not always the same) has mercifully passed with no incident more significant (but predictable) than Mrs Margaret Thatcher's rudeness towards the French. Not a single participant should be berated for not having read the whole of the 22-page communique he or she signed, but the document is nevertheless important: the heads of the seven richest governments sound more tentative than is their custom. That may be progress.

The economic issues, customarily dominant in these annual documents, are this time dealt with in a blend of *fortissimo* and *sotto voce* almost calculated to make cynics of us all. The overall message is that things are going well ("World trade developed rapidly last year", for example), that all kinds of past threats (chiefly to growth) have been avoided by cleverness. But there are also a few problems still to be dealt with — chronic trade imbalances (Japan and West Germany earn too much, the United States and — latterly — Britain too little), chronic budget deficits (the United States and Italy are the culprits), the threat of inflation (exported from the United States) and that of recession (caused by high interest rates) as well as the tendency towards trade protection. There is also the debt of the developing countries to worry about. Everybody agrees that the surpluses, deficits, debts and restraints should be eliminated in the interests of mutual development. Paradoxically, everybody feels free to sign, even though almost everybody is a transgressor in one way or another.

The rest of the Paris communique is more interesting. Unremarked, there are two paragraphs on the plight of Bangladesh and a brief mention of that of Yugoslavia. There are also separate sections on the global environment, drugs and AIDS, in the first two of which is embedded a version of a common paradox: those now most acutely aware of the dangers, mostly those whose representatives went to Paris at the weekend, are those who have done most to create the dangers. Western Europe and North America between them are thought to produce more than 40 per cent of 100 million tonnes of carbon dioxide a year; if Mr Mikhail Gorbachev's appeal at the weekend to join the club had not been as quickly rebuffed, the summit-goers might have boasted of more than 75 per cent. And the drugs trade would not flourish

were it not for the economic disparities that make it profitable for poor people to grow otherwise useless crops in the knowledge that people in rich countries will pay through the nose for what can be extracted from them.

Even those not invited to the Paris gathering (though some turned up for other purposes) will acknowledge that problems such as these cannot be made to go away by wish-fulfillment. It is also plain that, within the familiar framework, the only viable schemes for helping the poor countries of the world make progress are plans such as that put forward by the US Secretary of State, Mr James Baker (while still at the US Treasury) for using credit created by the World Bank and the International

Greenpeace — an apology

IN our leading article of 15 June (*Nature* 339, 491; 1989), "A shadow cast on a good cause", we referred to Greenpeace as an organization which harboured "terrorists" and which carried out activities which could fairly be described as terrorist activities. We did not intend so to describe Greenpeace and we accept that there was never any evidence to support such a suggestion.

Greenpeace has pointed out that a commitment to non-violence is one of its founding principles and that, since its inception in 1971, this has never altered. Greenpeace organizations are established in 22 countries throughout the world and nowhere have they advocated, condoned or organized any violent activities. We entirely accept the accuracy of these statements. Further, it is well-known that Greenpeace and its supporters and employees have themselves been the targets of violence, most notoriously the sinking of their ship, the *Rainbow Warrior*, and consequent murder of Fernando Pereira, a Greenpeace employee, by the French state.

We are happy to take this opportunity to apologize to Greenpeace, its officers and staff for the distress and embarrassment that we have caused them. We also today publish a letter (see page 180) from Peter Melchett, executive director of Greenpeace UK. At their request we have made a substantial donation to the trust fund established by Greenpeace to provide for the widow and children of Fernando Pereira. □



Monetary Fund to bridge poor countries' debts. The trouble with that arrangement is that it is slow. The time-scale of debt reduction may not be much shorter than that of global change. Now that the members of the Paris club (next year, it is Washington) have been persuaded of the possibility of the latter, should they not have a greater sense of urgency to clear their desks of the former? □

University follies

British universities are in for another upheaval, this time in the cause of competition and accountability.

AFTER eight years of rapid change, British academics have been looking forward to a period of recuperation. But they will be disappointed. Last year's education act may have emerged from the British Parliament innocent of the distasteful clauses that would have allowed the newly-created Universities Funding Council (UFC) to require that universities should be bound by formal contracts to provide educational services in return for the public funds which they receive, but the universities should have guessed that the government would push its new council as far as possible in that direction. This is what has now happened. The UFC seems bent on creating a market in university education, one that will have to be managed at the beginning, at least.

This, it seems, is how the scheme will function. Later in the year, each university will be sent a letter describing the new arrangements that will include a notional cost for educating people in different fields. Physicists, no doubt, will be more expensive than economists, but not nearly as costly as medical students. Universities will then be invited to bid for allocations of student places that the UFC will be willing to support, possibly increasing their chances (and their intake of students) by arguing that the economies of scale will allow them to teach more for less (per head). The scheme is to be introduced from the beginning of August 1991 (when the universities' financial year begins); the UFC hopes to manage the transition to a kind of market in student numbers within four years, ending in 1995. From the beginning of the period, the general subvention of research costs (perhaps 40 per cent of the total) will be separately identified and distributed in a discriminatory fashion — one favouring the universities more successful at research.

One obvious danger, a kind of educational Gresham's law, is that all the money will end up in the universities that teach students cheaply, but do little about their education. But the UFC has thought of that: account is to be taken of the quality of the education provided in different fields of study by various universities. Just how that may (or might) be done could be clarified by the letters to be sent out later in the year. The UFC seems to be hoping that, by then, a formula involving variables such as the opinions of external examiners, students' appraisals of their teachers and — possibly — the opinions of inspectors, will provide objective yardsticks of educational per-

formance. Academics already aware of the injustices of the use of citation indices in assessing research performance will give a hollow laugh, especially if they are near retirement age. Quite how the untested (and still unconstructed) yardsticks of educational performance should be used to modulate the numbers in different denominations thrown up by the separate bids for students of the universities may be a useful exercise for the few philosophers left in academic posts.

The new system, still only a declaration of intent, cannot but have a profound effect on British universities. One objective is to give universities an incentive to compete against each other for students — not in itself a bad thing — but the result of failure (or even success) will often be to skew institutions in directions that are educationally undesirable — too much (or probably too little) physics, too much accountancy and economics. The new system will also entail the *de facto* breakup of Britain's two federal universities (London and Wales), where it is unthinkable that colleges offering a universal range of courses will allow their chances to be miffed by head offices. There is bound to be a frightful row about the extra costs of Oxbridge teaching arising from the separation between the colleges and the universities. And the competition will not be really free: London costs are higher than elsewhere, which will have to be allowed for.

The most serious objection to this scheme is not that it is still half-baked, or even that competitions within the genteel system of British universities is in some way wrong, but that if competition is considered necessary, this is the wrong way in which to bring it about. If British universities were not almost entirely dependent on state funds, they would already be competing cheerfully, and would have reached some kind of dynamic equilibrium. So why not simulate that state of affairs? Two models have been much discussed. Notional sums of money could be attached to students in different fields, who could then pick and choose among the institutions offering them student places, or the institutions could be given the equivalent of an endowment (an assured income) and encouraged to spend it as they chose. Each has deficiencies, but none so serious as the basic error of what the UFC is now attempting — the micro-management of British universities with the help of yardsticks of which the only certainty is that they will (when designed) be inappropriate to higher education.

After such a long period of deprivation, British academics are not in a mood to answer back. But they should. The committees on the assessment of academic performance will soon be at work, manned dutifully by academics. Why do they not refuse to do the work, on the grounds that whatever yardsticks are devised must make for nonsense and injustice? There is a view that people with expertise in, say, chemical warfare should not work at the development of chemical weapons. Why should academics, even those who favour competition, assist a process that must undermine the health of British universities, and the welfare of those who work in them. □

Plagiarism charge casts shadow on peer review

- Referee accused of abusing privilege
- Author plans appeal against NIH ruling

Washington

A SERIOUS abuse of the peer review system has been alleged by an investigating committee of the National Institutes of Health involving a paper published in the journal *Science*. A short announcement in *Science* last week revealed that an NIH investigation found that a paper published in 1987 had plagiarized information published two months earlier in the *Proceedings of the National Academy of Sciences (PNAS)*.

"The wanton abuse of the peer review system with the clear intention of personal aggrandizement, attacks the very fabric of the scientific enterprise", says the report of the NIH investigation. "In the view of this panel, the charge of theft — plagiarism — is at least as serious a misconduct in science as outright fabrication of experimental data....".

The author charged with plagiarism is C. David Bridges, then at Baylor College of Medicine in Houston, Texas and now at Purdue University. His paper (*Science* **236**, 1678; 1987) is considered by NIH to have used information from a manuscript by Paul S. Bernstein, Wing C. Law and Robert R. Rando, all of Harvard Medical School (*PNAS* **84**, 1849; 1987).

NIH has recommended that Bridges be debarred from receiving federal funding for a period to be determined, be excluded from service on any Department of Health and Human Services' peer review committee for ten years and that NIH support for his current work should be terminated.

Bridges denies all the charges laid against him and intends to appeal NIH's findings. No blame was attached by NIH to Bridges' co-author and senior technician, Richard A. Alvarez.

The paper by Rando and colleagues reported the activity of an enzyme, isomerase, that completes the visual cycle that regenerates the visual pigment, rhodopsin, in the vertebrate eye.

The dispute began in July 1986 when Bridges was sent a copy of the Rando manuscript to review for *PNAS*. Eight weeks later Bridges returned the paper, excusing himself from reviewing it on the grounds that he was already engaged in similar work.

In November, Bridges submitted to *Nature* a paper that also claimed to demonstrate a retinol isomerase. But *Nature* declined publication. Bridges then revised the paper and submitted it to *Science*, which accepted it for publication in April 1987, just over three weeks after the

Rando paper was published in *PNAS*.

At about the same time, Rando became aware of Bridges' work, through an abstract prepared for the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO). He saw its similarity to the research he described in his *PNAS* paper and was able to find out that Bridges had been sent the paper for review, and that Bridges had a paper in press at *Science*.

The dispute could have ended at this point, for Rando did not accuse Bridges of plagiarism, and Bridges claimed (as he still does) that his own experiments began months before he saw Rando's manuscript. All Rando asked was that Bridges acknowledge his research. At the annual ARVO meeting, Rando met Bridges and they agreed on modifications of the *Science* paper, then in galley proof, that would have clearly acknowledged the priority of Rando's *PNAS* paper.

But according to the NIH report, Bridges "unilaterally modified the agreed-upon changes" and "attempted to claim priority for the discovery in news releases (instigated and authorized by him) which were devoid of any mention of the the Rando publication". Only then did Rando talk to Alvarez, Bridges' co-author, and hear that Bridges had not begun experiments on the isomerase until August 1986, after Bridges had received Rando's manuscript to review. When Rando passed this news to Baylor College, it was decided to set up a committee of investigation. The committee's report, issued in May 1988, triggered a full NIH investigation.

The NIH panel was at first frustrated in its efforts to tackle the key issue of when Bridges began his experiments. It criticized the quality of *Science*'s review process, finding the paper contained "internal inconsistencies, incomplete data, and misinformation". The panel was "dismayed by the absence of unequivocal documentation" — all the research records were facsimiles drawn from computer records, and the dates on them could easily have been altered.

But the panel found that a key piece of evidence remained: the shipment records for the radioactive retinol needed to conduct the experiments. The NIH panel found that the records "coincide with a chronology of experimentation beginning in August", after the Rando paper had arrived. Insufficient retinol was available to conduct the experiments earlier, in the panel's opinion.

PARTICLE PHYSICS

LEP in line for August advance

Washington

A LOW-energy positron beam successfully circumnavigated the ring of magnets constituting the Large Electron-Positron Collider (LEP), the new machine at CERN (European Laboratory for Particle Physics) in Geneva, Switzerland. At 20 GeV, the positrons were far below the design energy of 100 GeV, but this first test demonstrated that the magnets were all correctly aligned and that the high vacuum system for the ring was sound.

A similar test of the counter-rotating electron ring was scheduled for Tuesday of this week, and first physics experiments are planned for early August. David Lindley

The panel's judgement was clinched by an examination of the evolution of the research. When questioned by the committee, Rando was able to give "considered and empirically sound answers" for his choice of experimental conditions that included a low ethanol reaction mixture. But Bridges "did not provide convincing scientific justifications for the changes from his established methods", and "attributed to luck his asking for 'minimal ethanol'...".

The panel concluded that the successful procedure "was revealed to Bridges in its entirety through some intuitive leap (a rarity in experimental science)...or was plagiarized from the Rando manuscript". This logic and other inconsistencies in Bridges' protocol led the NIH panel to declare Bridges had committed plagiarism.

Could *Science* have done more to deal with the Bridges' case before publication? Rando says that he does not "have any complaints at all" about *Science*'s handling of the paper. He wrote to *Science* before the paper was published to say he had entered into an agreement with Bridges on changes to the paper. But he notes, "I did not say I suspected plagiarism".

Daniel Koshland, editor of *Science*, says that on the basis of the information the journal had at the time, "it would not have been fair to hold up the paper". As manuscript editors at *Nature* know well, complaints about incomplete citation of earlier work are common but generally of no great substance.

The case also brings up the much-debated question of who owns data gained in research paid for by federal funds. The NIH committee felt that it was incumbent upon Bridges to produce original data and was not convinced by claims that the data had been stolen or discarded. The investigatory panel stressed that it was a mistake for the Baylor College committee to think that the laboratory notebooks were Bridges' personal property.

Alun Anderson

Students can stay in US

Washington

THE US Senate passed a bill last week that would permit the 40,000 people from the People's Republic of China studying in the United States to remain in the country until such time as the administration rules that it is safe for them to return home. A similar bill in the House of Representatives could be put to a vote within weeks. But it is not yet certain that the measure will become law; some critics feel the action is too hasty and too drastic.

Bill Carroll of the National Association of Foreign Student Affairs (NAFSA), which has 6,500 colleges and universities as its members, says the measures could "jeopardize the future of international educational exchanges" by discouraging the government of China from sending students abroad. NAFSA is in favour of simply extending the length of time students may stay. The new bill would provide an automatic one-year extension of all visas held by Chinese students and then allow changes in residency status, possibly leading to permanent residence.

Chinese students who wish to come to the United States are still applying for visas at the US embassy and Chinese academics due to come here on study trips are also continuing to arrive. But despite the appearance of normality, arrests are still being reported daily in China for "actively supporting the spread of bourgeois liberalization". More than 2,500 arrests have been made.

Further arrests reported by the human rights group Asia Watch include 15 students, researchers and faculty; most were at institutions based in Beijing. Among them are Cheng Mingyuan, a professor at the Foreign Languages Institute in Beijing who had also been imprisoned during the Cultural Revolution while working for the Chinese Academy of Sciences; and Zhao Yiqiang, a teacher at Beijing Medical University and his wife. Trials and sentences publicly reported so far have been of workers, not students.

Although Chinese student organizations have been calling for harsh sanctions against China, and a bill passed by the Senate last Friday called on the president to review the whole range of economic ties between the United States and China, Chinese academics warn against too harsh a response. No US academic body has followed the example of the National Academy of Sciences and 'suspended' exchanges with China. Chinese academics in the United States say such moves may harm the very people they wish to help.

Universities will also be looking out for the safe return of Chinese students who went home during the summer vacation and should be back in the United States in September to resume their studies. At least one academic will not be returning. Liu Xiabao, a visiting scholar at Columbia University and a known dissident, was arrested in June after returning to Beijing.

Christine McGourty & Alun Anderson

BIOTECHNOLOGY

Cetus banks on interleukin

San Francisco

A MAJOR gamble by the embattled genetic engineering firm company Cetus finally bore fruit last week, as Denmark became the first nation to approve the California company's immunostimulatory drug interleukin-2 (IL-2).

In May, the European Community (EC)'s Committee for Proprietary Medicinal Products recommended the drug's approval, raising Cetus's hopes that other EC nations will quickly follow the Danish example. Cetus has paid out \$100 million over eight years to develop IL-2 as its flagship drug, banking on a vast market worth several times its investment stake. The drug, which Cetus will market as Proleukin, has been approved only for renal cancer. But human trials indicate that its benefits may extend to many other conditions, including melanoma, ovarian cancer, leprosy, hepatitis and AIDS-associated Kaposi's sarcoma.

On top of years of research investment, Cetus endured a bitter patent battle with competitor Hoffmann-La Roche Inc.

before the two companies reached a cross-licensing agreement last December. Even though this means sharing royalties, Cetus president Robert Fildes predicts that Proleukin will generate several hundred million dollars in sales. US approval of the drug is expected by mid-1990.

For a company with a stock price which has long been depressed and with only \$30 million of sales in the 1988 fiscal year, the Danish decision is welcome news. But more problems lie ahead.

Cetus has retained all marketing rights to Proleukin, breaking a tried-and-true industry tradition of licensing products to more established partners for marketing. The company will sell the drug through distributors or its wholly owned subsidiary, Amsterdam-based EuroCetus, putting an enormous strain on operating margins.

Fildes maintains that the decision is sound. He says the incremental cost to Cetus of going into Europe on its own is probably 25 per cent, which makes a lot more sense than settling for a 5 or 10 per cent royalty.

Robert Buderl

COLD FUSION

No new money from US government?

Washington

A PANEL of scientists asked by the US Department of Energy (DoE) to look into claims of cold fusion has concluded that the experiments have not presented "convincing evidence" for excess heat production and that no special programmes or new efforts in cold fusion research are justified. The panel, chaired by J. R. Huizenga of the University of Rochester and N. F. Ramsey of Harvard University, was convened by the DoE's Energy Research Advisory Board (ERAB). Its report has yet to be officially approved by ERAB, but no significant changes are expected before it is sent to DoE Secretary James Watkins.

Although members of the panel visited laboratories at the University of Utah and at Stanford and Texas A&M Universities, where electrolysis experiments producing excess heat have been reported, they saw no working cells, and concluded that in none of the claims was the precision of the calorimetric measurements sufficient to "persuasively demonstrate" the production of excess heat. The lack of fusion products in quantities consistent with the claimed power output also detracts significantly from the credibility of the claims.

The report does say that the low-level version of cold fusion, as indicated by the small neutron fluxes measured first by Steven Jones and his colleagues at Brigham Young University, may be of scientific interest, and that in some experiments are purported to generate excess heat there are sporadic temperature 'spikes' that merit further study. But neither of these phenomena holds promise for useful large-scale energy production.

David Lindley

NUCLEAR ENERGY

Food processors from Wackersdorf

Munich

THE Bavarian government on 4 July signed an agreement to allow the company Wilden KG to produce kitchen appliances on the site of the abandoned nuclear reprocessing plant at Wackersdorf beginning next spring. It will complement the solar energy cell factory that Siemens AG and the electric utility Bayernwerke plan to erect on the site. The West German government had invested about DM2,600 million (\$1,370 million) in the site before the project was abandoned this spring (see *Nature* 339, 413; 8 June 1989).

The 4,800-metre long, 3-metre high security fence, which was the site of dozens of demonstrations against the plant, will probably remain, said spokesman Wolf-Dieter Remmele of the Bavarian government. After all, he said, "Even private companies need security".

Steven Dickman

Offshore blast causes alarm

Tokyo

AN undersea volcano burst into life last week only a few thousands metres off the shore of a large resort city 100 kilometres from Tokyo.

The eruption, which broke through the sea surface near Ito on the Izu Peninsula southwest of Tokyo, is the first in history to occur so close to the main island of Honshu. It began in the early evening last Thursday and lasted only a few minutes but was witnessed by many in Ito and by millions on national television. Camera crews were in the city because a spate of earthquakes has rocked the area in the past few weeks.

The Izu Peninsula rides on the edge of the Philippine plate which plunges beneath Japan and is one of the most earthquake-prone areas of the world. The area has dozens of volcanoes both on land and under the sea but none is thought to have erupted in the past few thousand years.

Tremors began in the Ito area in late June and by last week more than 20,000 earthquakes had been detected, including two measuring 5.5 on the Richter scale which caused landslides and a few minor injuries. Some experts predicted that a major earthquake might be imminent.

Instead, after a brief lull in seismic activity, strange booming sounds were heard emanating from the ground in the neighbourhood of Ito on 11 and 12 July. The Volcanic Eruption Prediction Liaison Council interpreted the sounds as being

caused by rising magma. The next day, the eruption began.

Spectacular video pictures of the event were taken by a Maritime Safety Agency patrol vessel, the 2,600-ton *Takuyo*, which was surveying the area only 500 metres from where the eruption broke through the sea surface. Following a submarine explosion which rocked the vessel, the sea bulged upwards and turned black from a plume of volcanic material. Moments later, steam and smoke burst through the surface in an eruption about 100 metres wide and 30 metres high. Five minutes later the sea was calm again.

Only shortly before, the *Takuyo* had detected a mound about 25 metres high on the sea floor at a depth of about 100 metres at the site of the eruption. A subsequent survey revealed that the eruption formed a crater in the mound about 200 metres across.

Minor tremors continued to jolt the city of Ito, and several hundred residents living near the shore were quickly evacuated to higher ground because of fears of further eruptions and possible tidal waves. Police closed shoreline roads leading into the city and telephone lines to Ito were jammed by calls from anxious relatives. Earthquakes continued to shake the city on Friday and a plume of brown water was seen at the site of the eruption. The Volcanic Prediction Council is warning that further eruptions may be imminent.

David Swinbanks

IMAGE
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Moments after the *Takuyo* had passed the spot, the sea erupted. Inset map shows that Tokyo is not far away from the explosion, marked x. Photo: Kiodo News.

Australia's network

Sydney

AUSTRALIA is to set up an academic research computer network. The Australian Academic and Research Network (AARNet) will be funded by the universities, rather than by a separate agency as has been usual elsewhere. Each university will pay between A\$25,000 and A\$50,000 according to size and needs.

"In Australia there have been no government organizations with either the money or the interest to take on a project like this. Real network developments have really flowered in the last five years — Australia will now be a part of that," said Professor Ken McKinnon, chairperson of the Australian Vice-Chancellors' Committee, which gave its blessing to the plan last month. T. E.

Cut-price fingerprints

New Delhi

THE Centre for Cellular and Molecular Biology (CCMB), an Indian government laboratory in Hyderabad, hopes to provide DNA fingerprinting services to customers in India and abroad at a price much cheaper than the fees currently charged for similar service by British and American companies. It is the first biotechnology-based service commercialized by India.

CCMB director Dr Pushpa Bhargava DNA fingerprinting was used for the first time in India last month to settle a drawn out paternity case in Madras. The Bureau of Police Research and Development under the Ministry of Home Affairs will set up a \$6-million independent facility to exploit the technology for forensic science.

Services will be provided free for government agencies but a fee varying from \$30 to \$150 dollars will be charged for others. With only a handful of private companies in the world offering DNA fingerprinting services, Bhargava hopes that the low-cost Indian facility will attract orders from overseas. K. S. J.

Laid to rest

Munich

AFTER a six-month investigation, an independent commission has identified five slides, six paraffin-mounted tissue samples and several other bodily remains in the collections of the University of Tübingen medical faculty that might derive from Nazi execution victims. University leaders, supported by the Ministry for Science and Art in the *Land* of Baden-Württemberg, decided in January to bury all body parts that are even suspected to derive from Nazi victims. The commission determined that it is impossible in many cases to trace the origin of slides or body parts. In cases where there is doubt about the origins of the remains, they will be given a respectful burial at 'Burial Ground X' of the Tübingen city cemetery. S. D.

Seven go environmental

Paris

THE planned coincidence of France's bicentenary celebrations with the fifteenth annual summit of the seven most industrialized nations ended with a spectacular weekend, but an unsurprising summit. Under President Francois Mitterrand's patronage, many expected the seven (G7) to announce significant resolutions on the environment and on north-south relations. But no new measures were agreed.

Under pressure lobbying from four of the world's poorest nations, Mitterrand opened the summit with a plea for a north-south summit. But this was politely put aside, with Britain and the United States the least enthusiastic. Instead, the accent was firmly placed on east-west relations, with President Bush fresh from his visit to Poland and Hungary last week.

The biggest surprise of the summit was a letter from Mikhail Gorbachev to President Mitterrand, delivered on Saturday. The letter expressed a desire of the Soviet Union to take a full share in international political and economic issues, in effect a request to join the next summit to be held in the United States in 1990. The seven were unanimous in agreeing that the time was not yet ripe.

On the environment, the seven reinforced the 1987 Montreal agreement to cut production of chlorofluorocarbons by the year 2000, but declined to introduce any new initiatives on global environmental issues. Deforestation was identified with problems of Third-World debt, for which "greater realism" on the part of the commercial banks and proper economic strategies for debtor nations were seen as remedies. The problems of flooding in Bangladesh and desertification in Africa were singled out as urgent priorities. The seven called for a conference on Bangladesh before the end of the year, possibly sponsored by the World Bank.

The summit was inevitably swamped by the French festivities and the most piquant political events took place outside the meetings. While the seven richest nations

met in a luxurious suite at the top of the brand-new Arche de la Defence building, "The other economic summit" of researchers and economists from some of the poorest nations united at the down-at-heel Mutualité assembly rooms.

The high point of the weekend was an extravagant parade down the Champs-Élysées. But instead of break-dancing

CARBON DIOXIDE EMISSIONS

Japan seen in good light

Tokyo

THE United States, the Soviet Union and China are pumping more carbon dioxide into the atmosphere than any other nations. By the year 2030, their emissions will have doubled or tripled and output from developing nations will also contribute a large proportion of worldwide emissions. Japan's carbon dioxide output, on the other hand, will show only modest growth and will constitute a tiny fraction of world output by 2030.

These are the main results of an analysis of worldwide energy consumption released last month by a study group of Japan's Ministry of International Trade and Industry (MITI). The group concludes that the United States, the Soviet Union and China need to institute stricter controls of carbon dioxide emissions. This view is shared by other Japanese government agencies and gives an indication of the stance Japan is likely to take in international discussions of the problem of global warming caused by build-up of carbon dioxide in the atmosphere.

The study group on long-term global energy problems, an advisory group composed of academics, media representatives and members of private industry,

Chinese students, as originally planned, the parade was led by a solemn procession of young Chinese in mourning pushing bicycles and ringing bells to the beat of drums. And two leaders of the Chinese student movement came out of hiding to inaugurate a replica of the statue of liberty demolished by tanks in Tiananmen square. At the summit, the seven nations avoided sanctions against China, calling for political and economic reforms and greater openness.

Peter Coles

examined worldwide energy consumption and carbon-dioxide emissions in 1986 and made projections for 2030.

The United States is currently the world's leading source of carbon dioxide, accounting for 23 per cent of emissions, with the Soviet Union a close second (18 per cent) and China third (11 per cent). Developing nations (including China) also contribute a large proportion (26 per cent) and their emissions are expected to show a dramatic four-fold rise by 2030, accounting for about a third of a total world output of about 18,000 million tons (on a carbon weight basis) by that time.

Annual emissions of carbon dioxide (millions of tons of carbon)

	1986	2030	Av. annual growth (%)
World total	5,575 (100)	18,184 (100)	2.7
United States	1,299 (23)	3,257 (18)	2.1
Soviet Union	1,030 (18)	2,940 (16)	2.4
China	621 (11)	1,218 (7)	1.5
Developing countries	1,452 (26)	5,891 (32)	3.2
Japan	260 (5)	419 (2)	1.1
South-East Asia	146 (3)	532 (3)	3.0

Figures in parentheses indicate percentage of world share. China is included as a 'developing country'.

In contrast, Japan, despite its huge gross national product, accounts for only 5 per cent of current emissions, and, assuming a modest annual growth (about 1 per cent), this share will drop to 2 per cent by 2030. Hisakazu Kato of the Environment Agency attributes Japan's low carbon dioxide emissions to rapid adoption of energy-efficient technology and 'clean' energy (for example natural gas) following the oil crisis of the early 1970s.

In its projections for Japan, the study group assumes expanded use of nuclear power (which does not emit carbon dioxide). But there is growing opposition to nuclear power among the general public in Japan and expansion schedules may not be met. Furthermore, although energy consumption rose slowly between 1975 and 1985, consumption in recent years has risen by about 5 per cent per year because of increased public consumption. Thus the estimate of carbon dioxide emissions for Japan in 2030 (419 million tons) may be low.

David Swinbanks

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GREENHOUSE EFFECTS

Unilateral action urged

London

THE Energy Committee of the House of Commons this week advocated modest unilateral action to reduce British production of greenhouse gases, in the process deftly turning most of the special pleadings of the energy-producing industries.

The committee's report, *Energy Policy Implications of the Greenhouse Effect* (HMSO, £8.70), is based on the assumption that international action will follow quickly and on the argument that action should not wait on the removal of uncertainties about the scale of the greenhouse effect.

The committee, noting that its remit is merely to look into matters covered by the government's Department of Energy, has nevertheless taken a broad view, producing a useful summary of the state of research and of the outcome of last year's conference on the subject at Toronto.

While accepting that "draconian" unilateral action could be economically perilous, the committee notes that some kinds of energy efficiency may be economically advantageous. It says that Britain "should consider setting an example to others", repudiates the idea that "because one cannot do much, there is no point in doing anything at all" and quotes Edmund Burke as saying, "nobody made a bigger mistake than he who did nothing because he himself could only do a little".

But what? Taking account of the greater infrared absorption by methane and chlorofluorohydrocarbons (CFCs) than by carbon dioxide, the committee argues for the reduction of methane leakage from gas pipelines (400,000 tonnes in 1987), the legal restraint of petrol (gasoline) leakage from automobiles (originally meant to combat smog by reducing tropospheric ozone, which is a greenhouse gas) and continued tightening of nitrogen oxide emissions from automobiles.

Methane from landfill sites, equivalent in its greenhouse effect to 10 per cent of that of carbon dioxide released each year in Britain, could be used economically as fuel or, alternatively, the dry waste from which it derives could be incinerated economically and with benefit.

The committee has uncovered an anomaly in the tariffs charged by British Gas for supplies to industrial consumers which, it says, encourage inefficiency; it wants that state of affairs remedied.

Of greater potential importance is the proposal that natural gas should be substituted for other fuels (on the grounds that its energy content per unit of carbon is greater than that of coal and oil). The committee dismisses British Coal's objections, but acknowledges that British reserves of natural gas are estimated at

only 40 years of supply, and that there may persist economic objections to substantial imports. "Orimulsion" (an emulsion of crude oil in water) is mentioned favourably, as is a Canadian plan to ship 100,000 tonnes of hydrolytic liquid hydrogen to West Germany for use as fuel.

On nuclear power, the committee is agnostic on the view of the Central Electricity Generating Board (CEGB, the largest utility, still nationalized, but not for much longer) that it could commission two pressurized water reactor nuclear power stations a year in the early 2000s, but says it is unlikely that there will be a programme on such a scale, given public distrust of nuclear power and the impending privatization of the industry. But the committee believes it would be "sensible" not to run down the fast-breeder programme as now intended.

The case of a promising coal gasification plant originally mounted as a joint project between British Coal and CEGB, from which the latter has withdrawn, emerges as a minor scandal. Completion of the

SOVIET PSYCHIATRY

Grey marks from US group

London

US psychiatrists acknowledge that there has been much progress in the improvement of the practise of psychiatry in the Soviet Union, but believe there is still some way to go. This is the gist of a report, issued last week in Washington, by a 26-person delegation of US psychiatrists and lawyers visiting the Soviet Union in February to test claims that the abuse of psychiatry to punish political and religious dissent is a thing of the past. Soviet psychiatrists are anxious to confirm their reinstatement as members at the next Congress of the World Psychiatric Association in October.

The delegation, which was led by Bill Farrand of the US State Department and Dr Loren H. Roth, was allowed to nominate the patients it wished to examine. Originally, 48 were named, 37 of them then in hospital. By the time the delegation arrived 17 had been, or were on the point of being, discharged, 4 were not available (due to death, imprisonment, emigration to the United States and insufficient data) and several did not wish to be examined by the delegation.

Only 27 patients, 15 in hospital and 12 discharged, were examined. Of these, the delegation notes, 5 were hospitalized when, by Western standards, they did not warrant a diagnosis of mental illness. Two of these were confined under Article 70 of the Soviet Criminal Code (Anti-Soviet Agitation and Propaganda).

project is in limbo for lack of funds, which the Department of Energy has been urging should be found from overseas, but for which it has now promised to seek government support in the next budget.

For the short run, the committee comes down in favour of energy efficiency (but renewable sources of energy get favourable mentions), acknowledging that market mechanisms are probably insufficient to make much difference. On the proposed carbon tax, the committee says that this would be inequitable if not international, but that a European Community tax taking account of all forms of pollution related to energy production might be workable.

The influence of the committee's report is unlikely to be huge. In due course, the British government will have to respond, even though (as some now speculate) the Department of Energy will have ceased to exist by the time it is considered seemly to have done so. The chief significance of the overpriced document is probably that it reflects the eagerness of a group of politicians in a conservative country to be doing (or to be seen to be doing) something. □

One of the hospitalized patients had been admitted in December 1988 with a diagnosis of schizophrenia, after a long period of activity on behalf of human rights. Although the patient had been discharged in February, he had never had to be charged with crime or diagnosed as sick because he was still on the psychiatric register after an earlier admission.

The delegation notes that mild disorders in several of the discharged patients would have been insufficient to warrant compulsory hospitalization in the West, and that that the diagnosis of "sluggish schizophrenia" in political activists seems still to be commonplace. The delegation has not been able to establish whether past abuses were deliberate, or whether the psychiatric confinement of dissidents had ever been accepted as government policy.

The Soviet reply, also now published, accepts that the US report is a "serious attempt to analyse the current situation in Soviet psychiatry", but repudiates allegations that the US delegation was deliberately impeded in its work. (Bureaucratic bungling was given as the true cause.)

But the Soviet psychiatrists accept US criticism of the use of sulfazine and atropine comas, while denying that these treatments are now used punitively. They also maintain that the US delegation approached the investigation in a one-sided manner and had too narrow a view of what constitutes criminal liability.

Vera Rich

INSERM closes the file

Paris

"WISDOM and courage have prevailed", says Dr Jacques Benveniste in a letter to the French national newspaper, *Le Monde*, last week, following the decision on his career taken by Philippe Lazar, director-general of the national institute of health and medical research (INSERM) (see *Nature* 340, 89; 1989). The two evaluation committees that reviewed the work of Benveniste and his group at Unit 200 were impressed by the overall performance of the laboratory, but both recommended that Benveniste stop his controversial work on high dilutions. As is his right, Lazar did not endorse this recommendation in his four-page letter to Benveniste, but suggested that much of the controversy was due to *Nature*.

In his letter, Lazar says that the "unprecedented" decision by *Nature* to organize a laboratory visit after publication of Benveniste's paper, the "oddness" of the visiting panel, the "offensive content" of its conclusions and the "questionable ulterior justifications of the journal regarding its real motivations", constitute extenuating circumstances in Benveniste's case. Lazar expresses surprise at *Nature*'s decision to publish what he calls an "insufficiently founded" paper in the first place. In reply to a question at a press conference last week, Lazar said he would still advise French researchers to submit papers to *Nature*. "*Nature* made a false step *vis-à-vis* this article", he said, "but I hope to God they will not do this again".

Most of the French press has reported the outcome of the four-yearly review of Benveniste's laboratory. But there has

been little sign of the passions aroused last year, when accusations of "froggy-bashing" were abundant. Indeed, at the press conference, Lazar appealed to the press to "let Dr Benveniste get on with his work". Benveniste was advised by Lazar to avoid speaking to the press. But in his letter to *Le Monde*, Benveniste was keen to have the last word, accusing *Nature* of having hit first. *Nature*, he says, caused a "con-



Benveniste: perchance to dream?

siderable media stir", both within and outside the journal. "I followed", he adds.

The tone of the reports of the two evaluation committees is severe. The scientific committee's report, dated 4-6 July, suggests that the controversy surrounding Benveniste's work could "harm the credibility" of the overall work of his group. They say that Benveniste's interpretations were "out of proportion with the facts", and appear as "a laboratory curiosity to which satisfactory explanations have not yet been given and whose import will remain limited". They ask INSERM not to continue to fund high-dilution research.

Lazar has not asked Benveniste to stop work on high dilutions, although he made it apparent that this could be incompatible with the ethics of research that Benveniste must respect if his post is to be renewed at the end of the year. Such an injunction, he says, would be contrary to the freedoms accorded to a laboratory director. But he does ask Benveniste systematically to look for sources of experimental error which, according to Lazar, could explain the "unusual results". In his letter to *Le Monde*, Benveniste complains that this is what he has tried to do.

The affair, says Benveniste, will "finish badly, in the utmost banality", implying that foreign researchers will now take the glory if his results are supported. "I have sometimes fantasized", he says. "I did not then know that physicists who deal with infinity have the right to dream, but that soft savants like biologists do not! Now I know."

Peter Coles

Researchers win pay awards in States . . .

Washington

US GOVERNMENT researchers and scientific administrators will benefit from the executive-level salary rise proposed in a bill sent to Congress last week by President George Bush. The bill sets out pay rises of between 8 and 25 per cent for top federal employees, and creates a special salary category that would pay up to \$124,400 to workers in 200 positions requiring "specialized and critical skills".

The low pay offered by government positions compared with those in private industry has meant high turnover and difficulty in filling senior scientific and technical posts in recent years. Half a dozen top researchers have left the US National Aeronautics and Space Administration since the beginning of the year, giving low pay as one of the reasons. And many government agencies are regularly turned down by a dozen or more prospective employees before they can fill high-level research jobs.

Legislation to raise salaries was introduced by Congress earlier this year but was voted down because it would also have given legislators a politically controversial 50 per cent pay increase (see *Nature* 337, 588; 1989).

Carol Ezzell

. . . and in France

Paris

DISCUSSIONS between the French research minister and unions representing researchers in the state institutes, which began in March this year, have ended in a package of pay increases and promotion opportunities. Remarkably, both parties are apparently happy with the outcome of negotiations. The FF370-million (\$59.2 million) deal affects some 15,000 researchers and 25,000 technicians and administrators and comprises improvements in salaries, career prospects, recruitment and opportunities for transfer to teaching posts within universities.

To encourage recruitment of young researchers, the starting salary for a post within one of the state organizations will be raised from FF111,600 (\$17,856) a year to FF120,000 (\$19,200).

The number of doctoral bursaries, currently valued at FF84,000, will rise by 50 per cent from the present 1,900. Half of the new grants will be available from September, the remainder next year.

Career prospects have also been improved. The upper age limit for recruitment at the lowest research grade has been set at 31, but direct entry at the upper end of the basic research scale (*chargé de recherche I*) will become easier. There is a target of 1,000 new promotions by 1993 half of these by next year. In addition, directors of large laboratories will be eligible for a FF36,000 annual bonus.

Peter Coles

ANIMAL EXPERIMENTS

Taking advice from experts

Paris

THE French ministries of agriculture, of health and of research have now set up a national commission on animal experimentation, as embodied in legislation which came into force in October 1987. The commission comprises eight representatives from the ministries concerned with animal experimentation (research, agriculture, health, industry, defence, environment and education) and twelve experts drawn from both the public and the private sectors. The commission will advise the government on all questions concerning animal experimentation, but especially on animal husbandry, training of researchers and technicians and the possibilities of finding experimental models that do not require animals.

Peter Coles

DIOXIN AND HEALTH

Agent Orange study blasted

Washington

"MONUMENTALLY bungled or politically rigged" was how US Congressman Ted Weiss last week described a pilot study by the Centers for Disease Control (CDC) that tried to tackle the controversial question of whether wartime exposure to Agent Orange has damaged the health of Vietnam veterans.

Veterans' organizations claim that thousands of soldiers have suffered ill-effects from handling Agent Orange and from exposure in areas where the dioxin-containing defoliant was sprayed in order to destroy cover for Vietnamese guerrilla fighters. But the CDC pilot study ended by concluding only that it would be impossible ever to untangle the effects of Agent Orange from many other factors affecting health. Of 34,000 veterans who have applied for compensation for health problems claimed to be caused by Agent Orange, only five have been successful.

Weiss (Democrat, New York), chairman of the House of Representatives human resources and inter-governmental relations subcommittee was not satisfied by the explanations delivered by CDC at a congressional hearing last week. He claimed that political pressure from the White House had led to a study "designed to be inconclusive". He is now demanding a new study by an independent organization and may introduce legislation to provide funds from Congress.

In a two-hour session of aggressive questioning, Vernon Houk, who led the CDC study, explained at the hearing that he had decided a full study was not worthwhile because most of the ground combat troops who did not handle or spray herbicides were not heavily exposed to dioxin, and that military records were inadequate to identify a large enough number of veterans who might have been exposed in order to validate a full-scale study.

A separate study, in which high levels of dioxin were found in the blood and adipose tissue of veterans heavily exposed to Agent Orange was cited as evidence that CDC may have missed a heavily contaminated subset of veterans. Philip Landrigan, director of environmental and occupational medicine at the Mount Sinai School of Medicine, New York, says that CDC must "dig further into the massive records which exist".

The conclusions of the CDC also conflict with those from a study carried out for the American Legion, which found that Agent Orange was significantly dose-related to a variety of health problems including benign fatty tumours, adult acne, skin rash with blisters, increased sensitivity to light and increased percentages of spouses' pregnancies that resulted in miscarriages. But CDC, the Congress-

sional Office of Technology Assessment and the Agent Orange Working Group of the Cabinet Domestic Policy Council all claim that the American Legion study is seriously flawed.

Helen Gellband of OTA said at a separate hearing on Agent Orange held last week by the House subcommittee on hospitals and health care that most ground troops probably had relatively little exposure, and criticized the American Legion study for including any location up to 15 kilometres from the spray path while other data indicate that no herbicide would have travelled more than 2 kilometres from the spray line.

EUROPEAN SPACE PROGRAMME

Cap on German space budget

Munich & Paris

THE first shots were fired last week in what is likely to be a major budget battle at the European Space Agency (ESA) over how much more should be spent next year on the Hermes space shuttle and the Columbus space station. Last week, the West German government turned down Research Minister Heinz Riesenhuber's ambitious request for DM1,700 million (\$894 million) for space projects in 1990. Nevertheless, Riesenhuber received a sizeable increase of DM175 million to DM1,300 million for 1990, which will allow West Germany to continue support for the time being for Hermes, Columbus and the heavy launcher Ariane 5.

Ministers from the 13 ESA member states voted at The Hague in November 1987 to establish a \$12,800 million manned space programme by the year 2000. The three large projects form the core of the programme, but several optional projects, including telecommunications satellites, microgravity experiments and Earth observation projects have also been discussed. It is these optional projects that are in the greatest danger from future budget cuts by West Germany and other member states.

In the most immediate danger, said an official of the Research Ministry (BMFT), is the data-relay satellite ERS-2, which is supposed to be launched in 1996 and would link the proposed ESA polar platform with the Earth. The ESA council is expected in October to propose an 'enabling resolution' to construct ERS-2, and member states will have three months to respond. The Research Ministry expects Britain and France to veto the satellite. Italy, which very much wants to produce the optical communications technology involved, is expected to push for the satellite. ESA president Reimar Lüst said in March that he considered the

Gellband says that the CDC's final exposure assessment method was superior to the American Legion method and that the CDC's results were borne out by the results of serum dioxin testing.

While the scientific battle goes on, a court in May ruled in favour of Vietnam veterans over compensation for health problems caused by exposure to Agent Orange. The Veterans Administration (VA) had operated a strict rule that compensation can only be paid when a causal relationship is proved between a specific illness and Agent Orange exposure. But the San Francisco court ruled that Congress required only a "significant statistical association" between exposure and illness for compensation to be awarded.

Christine McGourty

project to be essential.

Even Hermes and Columbus may be stretched out over more years than originally planned as a way to cut costs. Riesenhuber has sought to protect himself from cost overruns by promising that no more than 22 per cent of the BMFT budget would be spent on space projects. A BMFT official admitted that this could lead to delays.

The ESA director of space transportation systems, Jörg Feustel-Büechl, warned at an aerospace conference in Bonn in May of further cutbacks in the Hermes and Columbus programmes. Hermes is already "at the lower level of criticality", he said, and would become "just a technology programme" if it were cut back further.

European industry would be the biggest loser if optional ESA projects were to be cut and the other projects delayed. But member states have also put their pride on the line. Riesenhuber signed an agreement in Washington last week with Richard Truly, administrator of the national Aeronautics and Space Administration, for the launch of a West German spacelab mission called D-2 by the US space shuttle in February 1992, two months later than originally planned. D-2 is seen as an important milestone on the way to the space station.

Meanwhile, ESA president Reimar Lüst has been named as the next president of the Bonn-based Alexander von Humboldt Foundation. Lüst will take office on 22 September 1989, although he will remain president of ESA until 1990. Lüst, who is 66, follows Werner Heisenberg (1953-75), Feodor Lynen (1975-79) and Wolfgang Paul (1979-89) as president of the foundation, which brings hundreds of foreign researchers to West Germany every year on generous fellowships.

Steven Dickman & Peter Coles

Animal experiments

SIR—Although I am not a neurobiologist and cannot judge critically the significance of the article (*Nature* 337, 265–267; 1989) referred to by Clive Hollands in his letter (*Nature* 339, 248; 1989), I am nonetheless as disturbed as he is about the level of suffering involved in the experiments the article describes.

Two developments are taking place in human thought that should make all scientists disturbed when they encounter experiments of this kind. The first is the growing realization, from neurobiological studies themselves as well as from other disciplines, that we share many of our cognitive and perceptual attributes with many members of the animal kingdom. This alone should give pause to anyone contemplating severely invasive experiments on sentient animals. The second is the philosophical analysis of the ethics of human–animal interrelationships. This relatively new aspect of normative philosophy is effectively undermining the cartesian view of nature that has for so long spuriously insulated the animal scientist from the ethical consequences of his or her actions.

These two developments are heightening the awareness of many biomedical scientists that each time they use a sentient creature in an experiment, they face an inescapable ethical dilemma, namely whether or not the benefits of the experiment can justify the suffering inflicted. The extent of this dilemma is arguably proportional to the capacity of the animal to suffer and is thus particularly acute where the overlap with human attributes is greatest, as in the higher primates.

In my view, because of these ethical considerations, Mr Hollands was justified in conflating animal experimentation with publishing, and why your editorial response (*Nature* 339, 324; 1989) was, at best, evasive. I contend that *Nature* can no more escape the moral responsibility when it publishes research involving animals than can the investigator. *Nature* has at the least a duty not to undermine the morality of the society in which it publishes. Indeed, it could be argued that it has a duty to promote it. The laws that govern the use of animals in experiments represent, even if only crudely, an ethical compromise between the arguments that on the one hand animal experiments are always evil and on the other that they are value-free. Deliberately to publish research from studies that fall short of the legal and hence ethical standards set by the journal's own country is a cynical evasion of those standards. It can be defended only if *Nature* takes the position that the present restrictions on animal experiments are themselves immoral, in which case it should say so openly. If it

does not, it should abide by them.

Publishing the results of unethical studies on animals is advantageous to the perpetrator and his career. It is specious to equate this with the coverage of demonstrations against governments, because this is done in the belief that free speech is a 'good' thing. Animal experiments that ignore humane guidelines are felt to be a 'bad' thing and publishing results from them, in effect, condones them. Would *Nature* publish results of experiments carried out unethically upon humans? Moreover, to argue that if *Nature* does not publish these articles others will is patently unprincipled.

DAVID G. PORTER

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Anonymous authors

SIR—John Maddox's article on peer review (*Nature* 339, 11; 1989) reminds me of a proposal made by physicist Alfred Schild in 1959: "All scholarly organizations should accept for publication in their journals only those papers where the author remains anonymous. This would result in fewer and better papers being

published, those where the author (Anon. PhD) has something of real value to communicate."

ANDRÉ BACARD

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Mortality

SIR—We wish to correct a statement attributed to our company in an article entitled "Japanese doctors keep quiet" (*Nature* 339, 409; 1989) which says: "A spokesman for Kureha Chemical Industry, the company which developed Krestin, agrees that mortality of patients was not examined". In fact, "mortality of patients", which we understand to mean "survival rate of patients by randomized clinical trial", was examined many times, and the results of such examinations have been published^{1,2}. The clinical trials show evidence of increased survival when Krestin was given to cancer patients.

TSUNEYASU NISHIHARA

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1. Ohno, R. et al. *Cancer Immunol. Immunother.* **18**, 149–154 (1984)

2. Torisu, M. et al. in *Basic Mechanism and Clinical Treatment of Tumor Metastasis*. p.623–636 (Academic Press, London, 1985)

A letter from Greenpeace

SIR—The accusation in your leading article (15 June) that Greenpeace is, or has ever been, a terrorist organization is completely inaccurate. You have informed us that you did not intend to describe us as terrorists and there is no shred of evidence to support this allegation. In fact the reverse is the true position.

Greenpeace was founded on the principle of non-violence. We now have offices in 22 countries, and more than 3 million supporters around the world. In our 18 years of existence, Greenpeace has never advocated or condoned violence, nor been responsible for a violent action.

The allegation is particularly outrageous because Greenpeace has itself been the victim of violence directed against our employees. In the early 1970s, a Greenpeace boat, the *Vega*, was boarded by French marines while sailing in international waters, protesting against nuclear tests, and two of the crew were beaten up.

In 1985, the Greenpeace ship the *Rainbow Warrior* was sunk by bombs placed by French government agents, and a Greenpeace employee, Fernando Pereira, was killed.

Greenpeace respects the principles of scientific debate that a respected journal such as *Nature* fosters. We play an active part in encouraging scientific research on

environmental issues, employing scientists directly, and through support of scientists such as those funded by Greenpeace at Queen Mary College, University of London, and through modest grants for scientific research. We hope to encourage scientists to play an ever more active role in the current debate on environmental problems. We recently publicized a statement supported by over 100 scientists, including 16 fellows of the Royal Society, which said that the nuclear industry is wrong when it says that nuclear power has an important part to play in reducing emission of greenhouse gases, and which concluded that "nuclear power is irrelevant to the prevention of global warming".

We acknowledge your apology concerning the words used in your leading article of 15 June, and the donation you have made to the Trust Fund Greenpeace established to help support Fernando Pereira's widow and children. We look forward to future scientific debate, and to disagreements over how scientific data should be interpreted, but we can never accept unjustified and baseless attacks on the non-violent nature of our activities.

PETER MELCHETT

(Executive Director)

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A point on the arrow of time

Huw Price

In *A Brief History of Time*, Stephen Hawking leaves a tantalizing gap in his explanation of the arrow of time in terms of his no-boundary proposal for cosmology.

ONE of the outstanding achievements of modern cosmology has been to offer some prospect of a unified explanation of temporal asymmetry, the 'arrow' of time. The explanation is in two main parts, running something like this. First, the various asymmetries we observe are all thermodynamic in origin — all products of the fact that we live in an epoch in which the Universe is far from thermodynamic equilibrium. Second, this thermodynamic disequilibrium is associated with the condition of the Universe very soon after the Big Bang, the essential point being that in the rapidly expanding Universe, gravity can create organization much faster than other processes destroy it. The stars, galaxies and other forms of organization in the present Universe are products of this early period. Such concentrated energy sources themselves make possible the kinds of asymmetric phenomena with which we are most familiar, such as life itself.

This fascinating story has recently been given some well-deserved publicity in Stephen Hawking's best-seller, *A Brief History of Time* (Bantam, New York, 1988) — well-deserved, not least, because Hawking himself is responsible for a considerable part of the story as it presently stands. Hawking's book is something of a scientific thriller. Despite its merits, however, I think it commits one of the worst sins of that literary genre. The last pages offer us a surprising dénouement, but fail to explain its most puzzling aspect. It is as if we are assured that the butler did it, without being told how he overcame the evident obstacles (that he was incarcerated in Wormwood Scrubs at the time, for example). If the omission goes unnoticed by the casual reader, it is simply because he or she has not been told enough of the story to see that the climax is so surprising. Such a reader is doubly deprived, for mystery is the heart of a good detective story.

Unpalatable dilemma

In the case of cosmology and temporal asymmetry, the mystery lies in the fact that the attempt to explain thermodynamic asymmetry in terms of the expansion of the Universe from the Big Bang seems to lead to an inevitable and unpalatable dilemma. Hawking's account serves to mark this dilemma, in that he describes how he was led to change his mind about which of the possible conclusions is the

right one. In the process, however, he seems to gloss over the difficulty that has long plagued the conclusion he finally opts for. So it is not clear whether he has found some way around this difficulty or whether he has overlooked it. Either way, some clarification seems in order.

The dilemma stems from the fact that the Universe need not always expand. If the density of matter is sufficiently great, gravity will eventually reverse the expansion and the entire Universe will collapse to a black hole. What would then happen to entropy? Would it decrease as the Universe contracted or would it go on increasing, to reach its maximum value in the final singularity? Both answers seem unsatisfactory, though for different reasons.

The problem with the former answer is that it seems to entail that all the ordinary time asymmetries would be reversed as the Universe begins to contract. Radiation would converge on stars, apples would compose themselves in decompost heaps and leap into trees, and humanoids would arise from their own ashes, grow younger and become unborn. These humanoids would not see things this way, of course. Their sense of time would also be reversed, so that from their point of view their world would look much as ours does to us. The difficulty really lies in managing the transition. They lie in our future, as we lie in theirs. Various sorts of paradoxes seem to arise in the middle. These are discussed in P.C.W. Davies's *The Physics of Time Asymmetry* (Surrey University Press, 1974), for example. Note that we cannot avoid the problem by supposing (as on present evidence may well be the case) that the Universe never contracts. Even if the Universe as a whole never re-collapses, there is now a strong case that parts of it do, as certain massive objects collapse to black holes. Does entropy decrease in these cases? If we say that it does, the same sorts of paradoxes seem to arise.

What then of the alternative answer, namely that entropy does not decrease as one approaches a future singularity? The problem with this answer is that it makes it mysterious why entropy does decrease in the other direction, as one approaches the singularity at the beginning of the Universe. The difficulty stems from the time-symmetric character of the physical theories involved. If these theories imply that entropy was low in the region of this

initial singularity, then, by virtue of their time-symmetric character, it seems that they should also imply that entropy will be low towards a final singularity. So if we reject that option, we seem forced to conclude that physical theory does not explain the low initial entropy of our Universe. We cannot explain temporal asymmetry, in other words, but simply have to accept it as an extra-theoretical 'initial condition'.

Boundary conditions

This, then, is the dilemma: either we have to admit reversal of the thermodynamic arrow of time in the case of local or universal gravitational collapse; or temporal asymmetry turns out to be inexplicable after all, because we cannot account for the low initial entropy of the Universe as a more or less inevitable consequence of our best physical theory of the Universe as a whole. The dilemma is particularly acute for Hawking, for he has an additional reason to avoid resorting to unexplained boundary conditions. For him their effect is not simply to make time asymmetry inexplicable. They also conflict with the spirit of his 'no boundary proposal', namely that one restricts possible histories for the Universe to those that "are finite in extent but have no boundaries, edges, or singularities" (*Brief History of Time*, page 148).

Hawking describes how initially he thought that this proposal favoured the former horn of the above dilemma: "I thought at first that the no boundary condition did indeed imply that disorder would decrease in the contracting phase" (page 150). He changed his mind, however, in response to objections from two colleagues: "I realized that I had made a mistake: the no boundary condition implied that disorder would in fact continue to increase during the contraction. The thermodynamic and psychological arrows of time would not reverse when the universe begins to recontract or inside black holes" (page 150). In an earlier article that he wrote for *New Scientist* (113, 46–49; 9 July 1987), Hawking describes his change of mind in this way: "I then realised that, although it was possible for the Universe to contract back to a smooth and ordered state, it was much more likely to contract to a very disordered state, because there are so many more disordered states. Thus the thermodynamic arrow of time will not reverse. It will continue to

point in the same direction".

This change of mind enables Hawking to avoid the difficulties associated with reversing the thermodynamic arrow of time. What is not clear is how he avoids the alternative difficulties associated with *not* reversing the thermodynamic arrow of time. That is, Hawking does not explain how his proposal can imply that entropy is low near the Big Bang without equally implying that it is low near the 'big crunch' (or in a black hole). The problem was to get a temporally asymmetric consequence from a symmetric physical theory. Hawking suggests that he has done it, but does not explain how. Readers are entitled to feel a little dissatisfied.

There seem to be three possible resolutions of this mystery. The first is that Hawking has found a way around the difficulty, but has not told us what it is. The easiest way to get an idea of what he would have to establish is to think of three classes of possible universes: those which are smooth and ordered at both temporal extremities; those which are ordered at one extremity but disordered at the other; and those which are disordered at both extremities. If Hawking is right, he has found a way to exclude the last class without thereby excluding the second class. Why is this combination the important one? Because if we cannot exclude universes with disorder at both extremities, then we have not explained why our Universe does not have disorder at both extremities — we know that it has order at at least one temporal extremity, namely the extremity we think of as at the beginning of time. And if we do exclude disorder at both extremities, we are back to the answer that Hawking gave up, namely that order will increase when the Universe contracts.

Illicit appeal

Has Hawking shown that the second class of universal histories, the order-disorder universes, are overwhelmingly probable? If so, then he has not yet explained to his lay readers how the trick was turned. What seems clear is that it cannot be turned by reflecting on the consequences of the no-boundary principle for the state of one temporal extremity of the Universe, considered in isolation. For if that worked for the initial state it would also work for the final state — unless of course the argument has illicitly *appealed* to temporal asymmetry, in applying some constraint to the initial state that it did not apply to the final state. This is an important point. When we are trying to explain temporal asymmetry, we are not allowed for example to put any weight on the idea that the Big Bang occurs at the start of the Universe. After all, how could we tell that we are not mistaken and that the Big Bang is not really the finish of the Universe? We must assume that the truth of the matter is

that it is neither, and that our ordinary inclination to treat it as the start is just one manifestation of the temporal asymmetry we are trying to explain.

Hawking thus needs an argument that excludes disorder-disorder universes (those with high entropy at both temporal extremities) without also excluding order-disorder universes (or, what comes to the same thing, disorder-order universes). As he himself points out, it will then be quite legitimate to invoke a weak anthropic argument to explain why we regard the ordered extremity thus guaranteed as an initial extremity. By virtue of its consequences for temporal asymmetry elsewhere in the Universe, conscious observers are bound to regard this state of order as lying in their past.

That is the first possibility: Hawking has such an argument, but hasn't explained to his lay public what it is. As I see it, the other possibilities are that Hawking has made one of two mistakes. Either his no-boundary proposal does exclude disorder at both temporal extremities of the Universe, in which case his mistake was to change his mind about contraction leading to decreasing entropy; or the proposal does not exclude disorder at either temporal extremity of the Universe, in which case his mistake is to think that the no-boundary proposal does away with the need for initial conditions in explaining temporal asymmetry.

The former mistake would be the more pleasing. For one thing, it would restore the global symmetry in time which Hawking originally saw as one of the attractions of the no-boundary proposal — a symmetry which is lost if contraction need not lead to decreasing entropy. More importantly, it would mean that Hawking's explanation of local time asymmetry would still be intact — a much happier conclusion than the discovery that the no-boundary proposal simply fails to deliver its promised benefits, at least in this respect.

What is more, the former mistake might itself have a nice explanation, in terms of the temporally asymmetric character of our ordinary view of the world. One of the manifestations of this is that we regard it as natural for physical systems to be governed by initial constraints, but as highly unnatural for them to be governed by final constraints. We expect events to be determined by their past, but not by their future. Accordingly, we find it much easier to make sense of a universe evolving from tightly constrained initial conditions, than of it evolving towards similarly constrained final conditions. So it would seem odd to say that the universal histories whose discovery led Hawking to change his mind about entropy in the contracting Universe — those that start with order and finish with disorder — are excluded because they violate a final

condition stemming from the no-boundary condition. It seems miraculous that the course of the Universe at a particular time could be bound by conditions many billions of years in the future.

However, this seeming oddity is surely just a manifestation of our ordinary asymmetric way of looking at time. If we look at things from an atemporal perspective, as we need to if we are to explain temporal asymmetry in a non-circular way, then the oddity vanishes. Initial and final constraints stand and fall together. If we are entitled to one then we are entitled to the other.

Breach of faith

To pay lip service to the need for such an atemporal perspective is one thing; to be faithful in practice is quite another. This being so, it is conceivable that Hawking's concession to his colleagues does rest on a breach of faith at precisely this point — on a failure to insist that final constraints are as legitimate as initial constraints in narrowing the class of possible world histories. To show that entropy decreases in a re-contracting Universe, we do not need to show that the initial constraints themselves entail that entropy behaves in this way — that they themselves so restrict the class of possible histories of the Universe. We need only to show that the initial and final constraints jointly restrict the possible histories in the appropriate way. Given that much, and of course a plausible argument for both the initial and final constraints, there is nothing mysterious going on. Only a lingering attachment to the ordinary asymmetric perspective makes this use of final conditions look in any way suspicious. (It is true that there is still the mystery of what happens 'at the changeover', when entropy changes direction. But Hawking presumably regards this as a surmountable problem, as he was earlier prepared to advocate this view.)

It may be unfair of me to suggest that Hawking's concession to his colleagues might rest on this sort of conceptual mistake. If so, I apologize, and can say in my defence only that I'm a philosopher, and philosophers and physicists have a longstanding tendency to under-estimate one another (as illustrated by Hawking's own gentle dig at twentieth-century philosophy in the concluding pages of *A Brief History of Time*). But I do not think that it is unfair to claim that there is a tantalizing gap in Hawking's popular account of his endeavour to explain the asymmetry of time. I will happily accept Hawking's verdict and go back to the analysis of language (or whatever we philosophers do these days) if only he will tell us how the butler pulled it off. □

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Is there a future for research?

Onerous and unprecedented regulation of research, and financial difficulties, have given many researchers the impression that fruitful applications will now come only slowly, but others believe they flow too thick and fast.

WHATEVER happened to the golden age of science, when research was assured of unstinted support? Has public enchantment with the research enterprise melted away and, if so, why? Is the pace of innovation now so much more rapid than that of human evolution by natural selection that bewilderment is unavoidable?

These were some of the questions taken as starting-points for a discussion last weekend, organized by the Ditchley Foundation at its jewel of a manor-house in Oxfordshire, on the future of science and technology. Most often, Ditchley conferences, which are strongly Anglo-American, are overwhelmed by diplomats and politicians (although technical subjects such as arms control and greenhouse warming get a hearing).

Last weekend's meeting was unusual because of the presence of many influential researchers (Sidney Drell from Stanford, Joshua Lederberg from the Rockefeller University and Sir Walter Bodmer from the Imperial Cancer Research Laboratories, for example), research administrators and science advisers (including Professor Henry Durand from Paris, Dr Geraldine Kenney-Wallace from Ottawa, Mr J.W. Fairclough from the British Cabinet Office, Dr William T. Golden from the New York Academy of Science, Dr Hans-Peter Lorenzen from Bonn and Dr V. Ramalingaswami, now at UNICEF), politicians (Dr Jeremy Bray, MP, and Mr John Brademas, the Democratic congressman now turned president of New York University), academics (Professor Ashton Carter from Harvard University, Sir John Kingman, vice-chancellor of the University of Bristol, Professor Joseph Nye from Harvard University and Dr Robert Rosenzweig, president of the Association of American Universities) and others, such as Mr Peter Benton (director-general of the British Institute of Management, Mr John Chowcat from the (British) Manufacturing, Science and Finance Union and Mr Leonard D. Schaeffer, president of Blue Cross of California. The chairman was Dr David A. Hamburg, president of the Carnegie Corporation of New York, which last year set up a commission on Science, Technology and Government. (This list is not complete.) The reporting rules are that one may say what was said, but not who said it, which means that what follows may be larded with hindsight.

So was there a golden age, and has it

vanished? People are inevitably divided. Some note that the world at large has always been sceptical of as well as enthusiastic for innovation, while even now-beneficent innovations have been tightly regulated in the public interest. Others remark that the belief, widespread after the Second World War, that everything had become possible, was infectious, but was dispelled by the concealment by governments and technical people themselves of the hazards of new technology.

But attitudes towards new technology generally seem to be curiously patchy. The world at large has taken motor cars to its heart and the unsung benefits of telecommunications satellites and weather satellites for granted, but is cool about the US shuttle and sceptical of the US space station, perhaps because it is a project without a purpose. There seems to be a general opinion that progress will eventually hang on international collaboration, but the importance of man in space remains disputable.

The reasons why France alone among countries generating electricity from uranium appears to have avoided anti-nuclear protests fascinate those from elsewhere; good management is the simplest explanation, although the Commissariat à l'Energie Atomique has only recently adopted the practice of giving news of anything untoward that happens at its nuclear plants in the hope of engendering a sense that not all accidents are catastrophes. But there are clouds on the horizon. The green party that did well in France as in Britain in last month's elections to the European Parliament is already calling for a halt to nuclear construction (the last reactors in the current French programme will be commissioned in 1994) and for a reduction of nuclear-generating capacity to make the export of nuclear electricity impossible. And, as one participant said, "Just wait until you have a major accident!"

The new biology is the chief source of public passion. The research community seems to have won no credit for the Asilomar conference and the voluntary moratorium on research that followed. Genetic manipulation and embryology have become the only fields in which enquiry may be forbidden (as distinct from research with animals, where it is the procedures that are regulated). Some plead that legislation in the field should be flexible enough to accommodate discovery

and the greater sense of security that may follow, but governments, responding to their electors' opinions, are determined that research will be done with 'care', even if that means that the pace of development is slowed. There seems a gulf of understanding between biologists and governments (not to mention the public) about the human genome project (which in due course will create ethical problems for genetic counsellors, but which meanwhile tends to be lumped in with genetic manipulation). It hardly helps that some describe the project as "biology's pork-barrel".

What remedies are there? Better public understanding is a goal for everybody, but there is much doubt of what is intended. The ideal would be an educational system producing literate populations of democratic voters, but the need is too urgent for those now in kindergarten to join their ranks. Meanwhile, secondary schools, even if hampered from teaching as much science proper as they would wish, have a responsibility to give students a sense of what science and technology are like — that understanding is always incomplete and that its application is almost always followed by unexpected consequences.

Estimating and evaluating risk requires special attention. One speaker (a mathematician) warned that calculations of the risk of accidents in, say, the operation of nuclear reactors yielding estimates such as 'one in 10^7 ' should be generally disbelieved, on the grounds that the probabilities multiplied together in the process are not usually as independent as they should be. More practically, seeking to make machines safer by elaborating parallel control systems may induce a sense of complacency among operators.

But public understanding is not the only need. The research community's reputation for probity may be damaged by the recent flurry of scandals about dishonest publications. Some pleaded that the seriousness of this danger should be more openly acknowledged. Researchers as 'false prophets' present more difficult questions: the benefits of nuclear energy and of the Strategic Defense Initiative may have been oversold (and the claims on behalf of cold fusion may yet leave a black mark on public opinion), but it will be a bad business if people's enthusiasm for their research and its significance must be self-censored to be sober. Is not science also fun?

John Maddox

Phase transitions in the mantle

Raymond Jeanloz

THE phases of minerals at high pressure are of evident interest to those studying the physics of the Earth's interior. Thus the direct observation in laboratory experiments of a new phase of silica stable in conditions appropriate to the lower mantle, reported by Tsuchida and Yagi on page 217 of this issue¹, is bound to be noted by many. Although the volume of the new phase is only 1 per cent less than that of stishovite, the main silica phase in the mantle, its occurrence could have a dramatic effect in mantle physics.

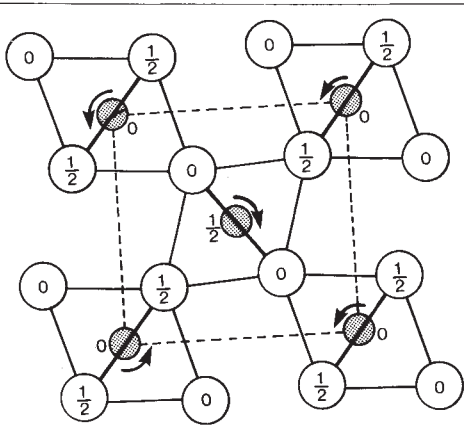
existing throughout the Earth's lower mantle. Their experiments are based on *in situ* X-ray diffraction through a high-pressure diamond cell; they use laser heating to synthesize samples at temperatures comparable to those of the mantle. In this way, they prove first that stishovite is stable to 100 (± 8) GPa, and second that at higher pressures it converts to the CaCl_2 structure (see figure) with a volume decrease of less than 1 per cent. The breakdown of the rutile structure occurs at much lower pressures than had been

structure, it might well dominate the mineralogical constitution of the deep mantle. Instead, Tsuchida and Yagi confirm the sense emerging from more recent studies¹⁵⁻¹⁹ that these ultradense phases are not stable at mantle conditions; they also show that previous identifications of post-stishovite phases for silica are either erroneous or represent metastable products formed on quenching²⁰. Thus, the fact that the rutile and CaCl_2 structures of silica are stable to the pressures of the lowermost mantle supports the current view that the perovskite phase of $(\text{Mg}, \text{Fe})\text{SiO}_3$ makes up the bulk of our planet's interior⁴: assemblages based on oxide phases (mainly $\text{SiO}_2 + (\text{Mg}, \text{Fe})\text{O}$) are simply insufficiently dense to be stable.

One might then reach the negative conclusion that the rutile/ CaCl_2 transformation in silica is not important for the Earth's interior, except that recent experiments suggest otherwise. Specifically, Knittle and I have shown that liquid iron reacts vigorously with crystalline silicates and oxides at the conditions of the lowermost mantle, with crystalline silica being one of the phases produced²¹. Therefore, reactions between the silicate mantle and the liquid iron alloy of the outer core are likely to produce silica near the core-mantle boundary. An extrapolation of Tsuchida and Yagi's data to lower mantle temperatures indicates that the phase transition in silica could occur at about 120 GPa, corresponding to a level about 270 (± 160) km above the core-mantle boundary. This possibility is especially interesting because the transformation should be associated with a vanishing of the c , rigidity modulus, and may therefore appear as an anomalously low seismic velocity^{7,9,22}. I estimate there is a 10–30 per cent softening of the average shear modulus of a stishovite aggregate upon transformation, suggesting a variation of 0.2–1.0 per cent in the shear-wave velocity of the lowermost mantle assemblage. Tsuchida and Yagi's result may thus be significant for understanding the D'' region, the heterogeneous, and seismically anomalous layer making up the bottom 200 km of the mantle²³. □

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The CaCl_2 structure, viewed in projection on (0 0 1), is a slight distortion of the rutile structure¹⁰. Shaded and open circles represent Si and O ions, respectively, and heights are given as fractions of the c unit cell dimension (the unit cell is indicated by the dashed line, with the a and b dimensions set equal for convenience). The transformation involves rotations of the SiO_6 octahedra towards a close-packed configuration, as shown by the arrows (each anion and its nearest cation neighbours are coplanar in the rutile structure). The rotation is identical to the motion of the B_{1g} Raman-active vibration of rutile; softening of this vibrational mode leads to an elastic instability of the $c_s = (c_{11} - c_{12})/2$ rigidity modulus.



Stishovite is the rutile-structured, high-pressure phase of silica (SiO_2). It is rarely found as a mineral in nature, but its significance to Earth sciences and crystal chemistry has far exceeded what its natural abundance might indicate. The importance of stishovite was partly evident upon its discovery in the laboratory²: it was the first example of a densely packed, octahedrally coordinated silicate (a crystal structure with six O ions surrounding each Si). This is in contrast with the tetrahedral (4-fold coordinated) structures characterizing the silicate minerals of the crust, yet it is now recognized that octahedral silicates make up the bulk of our planet's interior^{3,4}. In addition, the observation of stishovite in natural rocks has been crucial to documenting the importance of meteoritic impact as a major geological process on planetary surfaces^{5,6}. That is, the conversion of quartz, the common low-pressure form of silica, to stishovite proves that (impact-induced) high pressures have occurred at the surface, because stishovite is formed only above 7–10 gigapascal (GPa; that is, above pressures of 70,000–100,000 atmospheres). Now, the discovery by Tsuchida and Yagi that stishovite breaks down to a new crystalline structure at much higher pressures turns out to be equally intriguing.

The authors document in this issue¹ what happens to stishovite as it is taken to pressures of 30–124 GPa, the conditions

expected from lattice-dynamical calculations and from extrapolations of Raman spectra to 45 GPa. In the latter, the transition corresponds to the B_{1g} vibrational mode becoming soft (approaching zero frequency; see figure)^{7,9}. A qualitative inference is that the transition is associated with an increased ionicity (reduced covalency and hence less directionality) in the Si–O bond under pressure¹⁰.

These results will confound many geophysicists who have spent several years postulating the existence of post-stishovite phases that are much more dense at mantle pressures. These ideas were based on high-pressure experiments with analogue fluorides and oxides (MnF_2 , SnO_2 and TiO_2)¹¹⁻¹³, as well as on theoretical arguments¹⁴. Were there a more-dense phase of silica, such as in the fluorite or $\alpha\text{-PbO}_2$

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A missing link for seeds?

Bill Chaloner

In the history of life on land, with the interplay between plants and animals that it records, it was plant evolution that set the pace. The first step in this process was the 'greening of the land' as terrestrial habitats were colonized by vascular plants; they made the land habitable for animal life by offering a shaded, moist environment with built-in primary productivity, into which first the arthropods and later the vertebrates came ashore. The second step was the evolution of the seed — a pre-packaged food parcel evolved as a store to give the plant embryo a start in life, but subsequently exploited by herbivores for their own nutrition. The discovery by Galtier and Rowe, reported on page 225 of this issue¹, of an entirely new type of early fossil seed in the Montagne Noire deposit in France, raises important and unexpected questions about the details of this major step in land-plant evolution.

During the past 20 years there have been many significant additions to the fossil evidence, providing a picture of the evolutionary stages leading up to the first seed^{2,3}. There is now a well-documented record⁴ of the spore-bearing ancestors (the Progymnospermopsida) which gave rise to the earliest gymnosperms. There is general agreement that these were the stock from which all living seed-bearing plants (both gymnosperms and angiosperms) ultimately arose. There are still disagreements about details, such as whether the seed evolved only once (monophyletically) or whether the seed appeared

independently more than once (polyphyletically) from different groups within the progymnosperms⁵.

The origin of the seed was an important breakthrough in land-plant reproductive strategies. Heterosporous plants of the late Devonian reduced the number of megaspores in each sporangium to culminate in a single, very large surviving

occupies an intermediate position between, say, the condition shown in *Bensonites* and *Archaeosperma* in the evolutionary series in Fig. 1.

Does this newly discovered fossil seed-precursor then represent a 'missing link' in seed evolution which survived into the early Carboniferous, after the appearance of true seeds with complex pollen-trapping structures? Or is it simply an immature stage of development which would eventually have formed some pollen-trapping structure like that seen in contemporaneous seeds of the early

Carboniferous? Of these two possible explanations, the latter must be rejected as the well-preserved tissue at the seed apex is very clearly mature. Some other pollination (or fertilization) strategy must have been involved, but what form it took can only be guessed at. Maybe this would-be seed simply fell into its swampy habitat and its egg was directly fertilized by sperm released into a surface water film. This would make the behaviour of

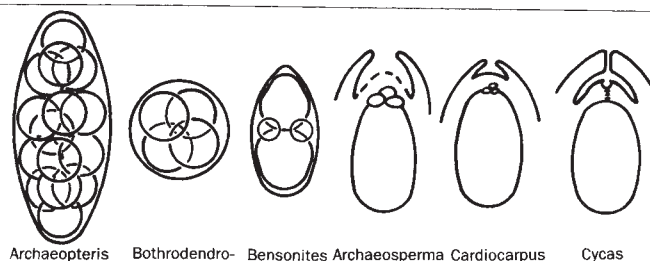


FIG. 1 A series of Devonian and Carboniferous megasporangia and seeds representing possible evolutionary stages in seed evolution, showing progressive reduction in megaspore number, and enclosure of the seed megaspore (from ref. 2).

megaspore forming the central structure of the earliest seeds⁶ of the end-Devonian/earliest Carboniferous (see Fig. 1). Then, instead of sexual reproduction involving motile sperm swimming through soil moisture to fertilize an egg in a megaspore lying on the ground (as in living *Selaginella*), the fertilization process was internalized. This gave plants greater independence from moisture in the environment in effecting their reproduction. Pollen borne on the wind was trapped by these early seeds, probably in an exuded moisture drop (as in living conifers), and drawn into a pollen chamber. This meant that the egg enclosed in the seed could be fertilized while still attached to the parent plant. The developing embryo could then be supplied with a food store before being shed, still inside the seed.

The discovery reported by Galtier and Rowe¹ of a new type of early seed-like structure in lower Carboniferous rocks (see Fig. 2) raises important questions about how the first seeds evolved. Hitherto, all known early fossil seeds show clear evidence of a fairly elaborate pollen-trapping mechanism at the seed apex (for example, *Cardiocarpus*), although their structure is in other respects significantly different from that of living gymnosperms. The seed-like body reported by Galtier and Rowe (which post-dates our earliest record of seeds only by a few million years) is surprising in having no pollen-trapping structure, or evident access for pollen to the female gametophyte. This is despite its preservation being remarkably good, in that it retains all the internal details of its cellular structure preserved three-dimensionally in silica. This fossil

the whole structure more like the megaspore of a heterosporous plant, such as some of the living water ferns, rather than constituting a true seed. The further possibility exists that pollen was caught on the outer surface of the seed apex, rather as pollen lands on the stigma of flowering plants. A pollen tube could then have carried the sperm to the egg (siphonogamy), as in most living gymnosperms.

As with many fossil discoveries, Galtier and Rowe's seed-like body raises more questions than it answers. It certainly encourages re-thinking the question of seed monophyly, and may be seen to point to a polyphyletic origin. It is also a salutary reminder of how little is known about important details of early seed-plant fertilization processes, and how much the fossil record still undoubtedly holds. Further search for other early seeds in the Montagne Noire deposit, and others at the same or earlier horizons, will undoubtedly contribute further to our knowledge of this critical phase of land-plant evolution. □

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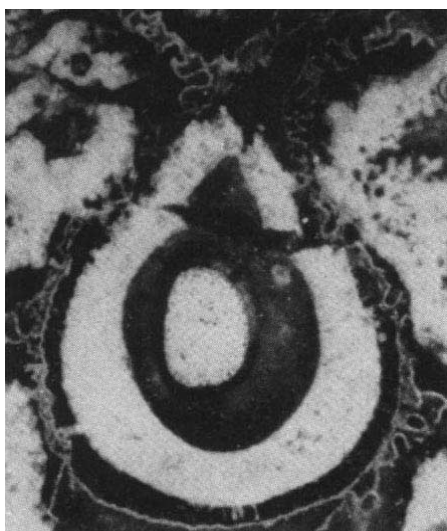


FIG. 2 New primitive seed-like structure from the early Carboniferous of France — oblique transverse section through integument lobes. (Courtesy of J. Galtier.)

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Motility factors on the march

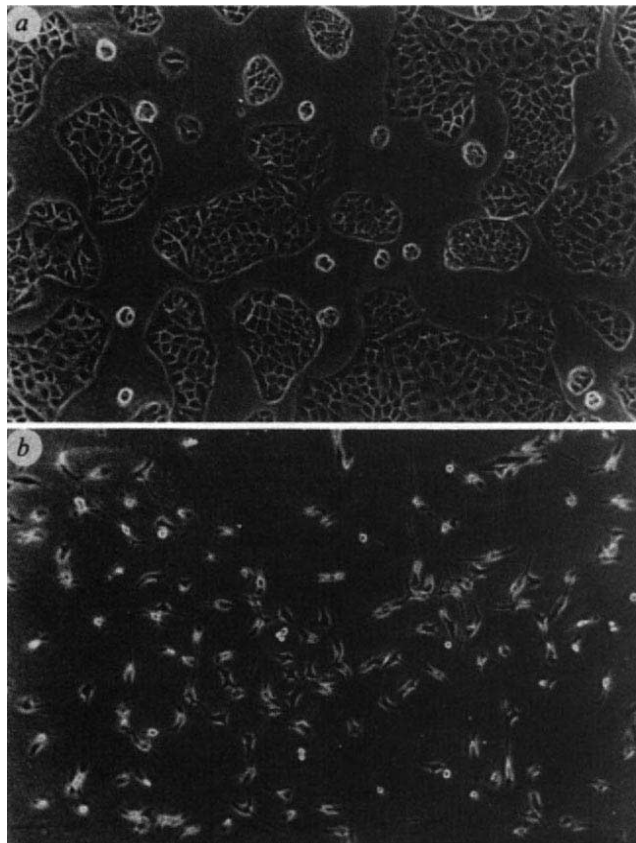
R.M. Warn and P. Dowrick

WHAT is the role of motility factors in the regulation of cell movement? The purification of two of these proteins, scatter factor, to be described by Gherardi *et al.*¹ in the July 31 issue of *Proc. natn. Acad. Sci. U.S.A.*, and the migration stimulating factor of Grey *et al.*², should help in understanding their roles in the regulation of motility of both normal and transformed cells. Motility factors are characterized as having significant stimulatory effects on cell movement but having no effect on cell growth. In this they are distinguished from growth factors, several of which also have effects on cell motility. The first evidence for motility factors was found by Yoshida *et al.*³ who demonstrated that certain tumour-derived cell lines secrete an agent that enhances the cell's own motility. Subsequently, various factors have been reported that have effects on motility; of these the best characterized are called scatter factor, migration stimulating factor and autocrine motility factor.

Scatter factor, as has been shown by Stoker and colleagues^{1,4}, is paracrine in action, being secreted by various fibroblast types mainly, but not exclusively, of embryonic origin, and also by some transformed derivatives of these cell lines. Arterial smooth muscle cells also produce an identical or similar molecule⁵. Scatter factor acts on epithelial cells such as several kidney lines, mammary gland cells and keratinocytes of various origins⁴, breaking up contiguous cell sheets, causing the loss of the cell junctions. The cells then spread out, show membrane ruffling, protrude and retract conspicuous processes, and move in a random, chaotic manner. In a Boyden chamber assay, single isolated epithelial cells show random cell motility and also migrate up a concentration gradient of scatter factor⁶. This demonstrates that the factor has direct effects on cell movement rather than stimulating mobility by an indirect effect. Fibroblasts (whether or not they are producers of scatter factor) show no such response.

By contrast, both migration stimulating factor (MSF) and autocrine motility factor (AMF) act on the cells which produce it and are thus autocrine in action. Schor *et al.*⁷ have shown that MSF is produced by human fetal, but not adult, fibroblasts, to

cause the rapid migration of producer cells into collagen matrices. In this assay, non-producing adult fibroblasts migrate much more slowly. An exception to this finding is that tumor-derived fibroblasts, and also skin fibroblasts obtained from patients with familial breast cancer, frequently show the fetal rather than the adult migration pattern in the collagen matrix assay⁸. In their new report², Grey *et al.* show that



Canine kidney cells grown *a*, without and *b*, with scatter factor.

MSF also is secreted by cells of this origin.

AMF was originally purified from conditioned medium (lacking serum) obtained from a human melanoma cell line by Liotta and co-workers⁹. A similar factor has also been isolated from breast carcinoma cells¹⁰. When it is added to cultures of producer or responsive fibroblast lines in Boyden chambers, cells show an increase in both chemotactic and chemokinetic motility. It is not yet known whether any normal fetal fibroblasts

secrete AMF. Cells of the mouse NIH-3T3 embryonic line do not release AMF, but they do so copiously after transfection with *ras* oncogenes.

All three factors are heat-labile proteins of similar relative molecular mass: 62,000 for scatter factor; 70,000 for MSF and 55,000 for AMF. Both scatter factor and MSF are probably glycosylated. All the factors are specific for cells of a particular tissue type, but it is not yet clear whether they are members of a family of proteins or a heterogeneous collection with similar effects on motility.

How do motility factors stimulate cell movement? Pertussis toxin blocks the action of AMF, suggesting that a G-protein (or proteins) may transduce this particular signal, as is thought to occur for the stimulation of white blood cell motility¹¹. Agents which inhibit the adenylate cyclase pathway, however, have no effect on AMF. AMF induces the formation of distinct pseudopodia before cell migration begins¹². The pseudopodia contain prominent F-actin microfilament bundles and have significantly enhanced concentrations of the receptors for the matrix components laminin and fibronectin. Thus AMF causes changes in the organization of the cytoskeleton and of molecules interacting with the substrate matrix. It seems likely that AMF (and possibly the other motility factors) act directly on the responsive cells via surface receptor molecules and mediate the ability of the cells to bind to the substrate matrix. In turn, such changes would affect the organization of the cytoskeleton.

Clearly, the use of purified motility factor will be a useful new approach in dissecting the molecular changes that occur when a cell becomes motile, in particular during embryogenesis, which is surely where motility factors have their normal roles, and in cancer. AMF is secreted by several cell lines originally derived from metastatic tumours. It could be that the development of aggressive metastases which migrate into and through adjacent tissues, blood vessels or lymph nodes is

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accompanied by the secretion of AMF or other similar molecules by the tumour cells in large enough quantities to enable migration to take place. If this turns out to be the case, therapeutic possibilities open up, particularly that of inactivating AMF or its putative receptor by antibody blockade.

The relationship of MSF to familial breast cancer (and possibly other tumours) is also intriguing. How a dysfunction of fibroblast motility affects the breast epithelium is unclear: MSF may act primarily on hyaluronic acid synthesis and its effect on fibroblast migration may be a secondary consequence of changes in matrix organization. These changes in the extracellular matrix would then in turn affect breast epithelium metabolism. If a direct role for MSF is found in the aetiology of the disease, then ways to inhibit its action *in vivo* can be sought. Skin fibroblasts from about half the first-degree kin of people with familial breast cancer also show the fetal pattern of migration in collagen gels and may therefore secrete

MSF. Thus, screening relatives of people with breast cancer for MSF secretion by their skin fibroblasts may be a way to establish whether they are at risk.

Whether scatter factor is involved in the development of tumours also remains to be determined. This factor is secreted by various transformed fibroblast lines and a human teratocarcinoma line, so it is possible that it may allow fibrosarcomas to penetrate epithelia. There is no evidence yet that epithelial tumour lines make scatter factor: some of these lines are not responsive to the factor, but others retain sensitivity to it, so there may be complex interactions between epithelial tumours and the surrounding connective tissue. In addition, either one or more motility factor(s), or an as yet unknown factor, may mediate the tissue migration and remodeling which accompanies wound healing, and may well be of therapeutic value. □

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MAGMA CHAMBERS

In situ differentiation in magma

R. S. J. Sparks

THE cooling, crystallization and chemical differentiation of magma in subterranean chambers is one of the principal causes of the great diversity of rocks in the Earth's crust. A central issue concerns how crystallization occurs in magma chambers and what the consequences are for magma evolution. The relative roles of crystal settling and *in situ* crystallization along the margins of a magma chamber have been widely debated. Hitherto, it has been assumed that the two mechanisms would be geochemically indistinguishable. But on page 199 of this issue¹, Langmuir shows that this is not correct. He demonstrates that geochemical trends in magmas formed by *in situ* crystallization can be very different from those formed by equilibrium or perfect fractional crystallization. This is an important conceptual development as it offers the potential to distinguish different physical models in magma chambers. The new ideas also indicate that interpretations of igneous rock suites in terms of perfect fractional crystallization may sometimes be suspect.

Cooling of a magma chamber results in crystallization along the margins and in the interior which can be caused by convection allowing heat to be lost to the wallrocks. Crystals (whose composition differs from the melt's) nucleated at the margin can be swept into the interior to form a suspension or can remain at the margins growing *in situ*. Crystals in the interior can be sedimented out at the floor where further growth can occur *in situ*.

The relative importance of these possibilities is being hotly debated. For example Brandeis and Marsh² have argued that convection in magma chambers is weak so that most crystallization is confined to the margins. Others^{3,4} take the view that convection is important and that significant interior cooling, crystallization and differentiation results. There is also discussion on the relative importance of crystal settling and *in situ* crystallization^{5,6} because it has been recognized that when crystals grow, the evolved melt can convect away, allowing magma to differentiate — this is compositional convection.

In detail the margin of a magma chamber should consist of a mushy zone of crystals and melt separating entirely solidified rock from the interior magma. The thickness of this mushy zone could vary from negligible (the dimensions of a single layer of crystals) to hundreds of metres in the cumulate pile on the floor of a large chamber. Langmuir¹ has examined the consequences of crystallization within this mushy zone and the exchange of interstitial melt with the main body of magma. Two extreme cases are worth consideration. First, there is no exchange so the trapped melt solidifies and the interstitial crystallization has no role in magma differentiation. Second, one can imagine a thin mushy zone where exchange is totally efficient so that any melt released by crystallization is immediately mixed into the interior. This case corresponds to perfect fractional crystallization. The

interesting and more general case, however, is where the mush has significant depth and some part of the residual melt is exchanged with the interior. The principles of this situation have been investigated by Langmuir. These principles are simple but have far-reaching implications.

Consider as an illustration a crystal pile of plagioclase feldspar formed from a magma in which this is the only liquidus phase. Suppose at some depth in the mush the temperature has fallen to a value where olivine precipitates. The melt released from crystallization of olivine then exchanges with melt from the same chamber. Although the interior melt is in the plagioclase field, mixing with the more evolved melt will result in chemical trends which show the influence of olivine fractionation. Langmuir shows that profound departures from standard liquid lines of descent (evolution) are possible. The process also has implications for trace elements. Incompatible elements can be substantially enriched relative to major elements in comparison with enrichments generated by fractional crystallization. Langmuir cites several examples of igneous suites where these ideas explain features which were previously regarded as perplexing. For example, the enrichment of incompatible trace elements and the cryptic influence of clinopyroxene fractionation that is apparent in many mid-ocean-ridge basalts could be the consequence of *in situ* crystallization.

There is no need to dwell on the details of these results which are clearly explained in Langmuir's article¹. There are, however, implications which can be taken further. The *in situ* model requires a physical process to exchange interstitial melt with the magma chamber. Compositional convection is the most plausible mechanism. The process also implicitly implies disequilibrium. If the more evolved, lower-temperature residual melts migrate through the mush into the interior then they must be heated up and would attempt to re-equilibrate with surrounding crystals. The residual melt must ascend fast enough to avoid significant thermal and chemical re-equilibration. These disequilibrium effects will further complicate the analysis of the mushy zone. The prospects are promising that the distinctive effects of *in situ* crystallization can be recognized from geochemical data and that these observations will help our understanding of the mechanisms operating in magma chambers. □

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Ice-age Amazon revisited

Paul A. Colinvaux

If I could fly to the ice-age Amazon in a time machine I should expect to find moist tropical forest in the lowlands, as there is now, but the familiar Amazonian trees living in unfamiliar communities for which there are no modern analogues. The most important parameter of the Pleistocene vector that formed these communities was a cooling of 6 °C, although precipitation was probably less than now. These ancient tropical forests were disrupted with the warming that brought on the Holocene. And it follows that what seems now like ancient undisturbed forest is an ephemeral product of the past few millennia, just like the forests of eastern North America. But this view is, of course, a hostage to fortune, necessarily made on the strength of too few data.

What the Amazon was really like during the ice ages of the Quaternary is still unknown and is the subject of some dispute. An opportunity to review what little is known was provided at a recent meeting*. The environment of the ice-age

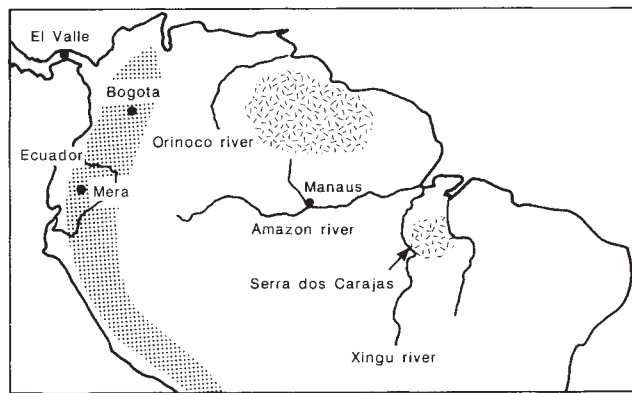
Amazon is particularly important because times of significant glaciation lasted five times as long as interglacials like the Holocene in which we now live. The warm phase of the Holocene is now nearly over, although the end of the latest glaciation was only 40 forest-tree generations ago. If the ice-age Amazon climate was indeed different from what it is today, then the modern rain-forest communities are younger than previously thought.

How important ice-age climates were for our understanding of the composition of modern communities has recently been shown in temperate latitudes, particularly for the forests of eastern North America. Thirty years ago, the prevailing hypothesis was that these forest communities were permanent, either resisting climatic change or migrating as intact communities. But this idea was finally falsified by accumulated pollen data demonstrating that the familiar North American forests are ephemera of the Holocene, produced over the past few thousand years by sequential migrations of individual species populations of trees dispersing out of the different centres of ice-age distribution. Oaks came by one route, hemlocks by another, and chestnuts and beeches by yet others¹.

How comparatively little is known of the Amazon past is illustrated by the fact that there is only one published radio-

carbon date of glacial age for the entire Amazon basin². For want of direct evidence, hypotheses to explain the high species richness of Amazonia have included climatic stability, intermediate disturbance and the refugial theory, which suggests that aridity gripped the Amazon lowlands with each glaciation to confine rain-forest communities to elevated regions with more rainfall³.

Empirical data from other continents and climate modelling studies suggest that there is increased tropical aridity during some parts of a glacial cycle⁴, but it seems



increasingly unlikely that the Amazon lowlands could have been sufficiently arid to destroy the rain forest. The observation most often cited to suggest aridity is that of irregular layers of pebbles seen in road cuts through lateritic regoliths over large areas of Brazil. One explanation for these 'stone lines' was that they represented pebbles that were washed out of a desert land surface⁵. M. Lichte (University of Göttingen) showed illustrations of stone lines in sections at the tops of slopes, from which he obtained a date, by thermoluminescence, of 20,000 years old. Examples of the lines are often seen inland from São Paulo. But the problems of how the pebbles were buried if they really are relics of a formerly devegetated surface was pointed out by H. Sternberg (University of California, Berkeley), who also noted the steep inclinations of many of the figured 'stone lines', which makes it most unlikely that their pattern could represent the position of the old soil surface. Sternberg followed Irion's conclusion⁶ that the stones were ancient concretions within lateritic soils.

The absence of convincing empirical evidence for dry climates in the ice-age Amazon lowlands leaves the concept of severe aridity only as a hypothetical isolating mechanism to explain disjunct patches of local endemism in forest communities. But B. Nelson (Amazon Forest Research Institute, Manaus, INPA) attacked the jugular of this hypothesis by re-examining

the evidence for local endemism in plant-distribution data. He used specimens of *Inga* in the INPA herbarium to map the intensity of collecting visits for this genus throughout the Amazon basin. The resulting map of collecting intensity coincides nicely with the proposed refugial areas. In addition, Nelson used the INPA collections of Chrysobalanaceae, a family whose apparent disjunct distributions have been used to argue for isolation of refugial areas, to test the idea that rare species have not yet been sampled enough to demonstrate range discontinuities. These preliminary data strongly suggest that sampling is indeed inadequate to demonstrate the absence of most species of Chrysobalanaceae, even from the most intensively sampled sites. These are preliminary data from a pilot study, but they do encourage the suspicion that patterns of local endemism in the huge Amazon basin have not yet been well established.

Data from the first section of the Brazilian Amazon radio-carbon-dated to glacial times, a core of lake sediment from the Serra dos Carajas near the Xingu River (see map), were reported at the meeting (K. Suguio, University of São Paulo; a team from the French

Institute for Scientific Research and Development (ORSTOM); and M. Absy, INPA). The core is from a shallow lake in savanna-like vegetation at 720 m elevation on the surface of an erosion plateau, but with rain forest within 5–20 km below the plateau. Frequent changes in gross composition of the sediments suggest a long history of fluctuating water level, probably reflecting changes in precipitation. Pollen data reveal intervals, one at about 15,000 and another at 30,000 years ago, when the total of Compositae, *Borreria* and *Cuphea* rose to more than 30 per cent from a background of about 5 per cent. This suggests episodes of reduced lake water levels, possibly a more arid phase of the local plateau savanna, or a combined temperature depression and changed moisture regime. These preliminary results give no reason to expect a climate so much drier than the present as to exclude rain forest from land below the Serra dos Carajas plateau.

With only this one incompletely studied section from Serra dos Carajas available for the Brazilian Amazon, reconstruction of ice-age conditions from empirical data must still be based on studies of the western Amazon along the Andean flank. The message here remains one of temperature depression coupled with fluvial disturbance. H. Hooghiemstra, P. Kuhry and K. Helmens (University of Amsterdam) reported their latest long sections from the Colombian Andes, reaffirming

*Global Change in South America during the Quaternary, São Paulo, 8–12 May 1989.

SUPERCONDUCTIVITY

earlier conclusions of descents of the altiplano vegetation suggestive of temperature depression of 6–9 °C in different parts of the last glacial cycle, together with lessened Andean precipitation between 20,000 and 12,000 years before present⁶. This picture is consistent with measurements of glacial descent in the Ecuadorian Andes reported by C. Clapperton (Aberdeen University) and S. Hastenrath (University of Wisconsin), as well as with estimates of forest depression from the Mera site at 1,100 m in Ecuador⁷.

The Mera site, although in lowland rain forest, is still relatively elevated. But pollen and phytolith (silica-cast) histories from old lake sediments at El Valle and Lake Yeguada in Panama (M. Bush, Ohio State University; D. Piperno, Smithsonian Tropical Research Institute) suggest descents of montane vegetation down to 500 m to elevations now occupied by tropical forests, implying a lowered temperature of 4–6 °C at sea level in the American tropics. Thus the available palaeoecological data and radiocarbon dates suggest that in parts of glacial cycles Amazonian climates were significantly cooler than today.

Meanwhile, there are various suggestions of river wander and flood in the western Amazon, both in the glacial maximum and since then. K. Campbell (Los Angeles County Museum) presented the latest version of his hypothesis that a great freshwater lake occupied the western Amazon in glacial times, held behind sediment dams across Amazonian tributaries and draining north to the Orinoco⁷. The importance of fluvial erosion in preventing competitive exclusion among forest trees⁸ was contested by J.F. Dumont (ORSTOM), who argues that highest diversity is found in elevated sites most protected from fluvial erosion. But the fluvial-erosion thesis⁸ can probably be extended to suggest that colluvial removal of surface soil is a significant destabilizing factor even in soils out of reach of immediate flooding.

The meagre evidence so far available thus points to a disturbed history for the Amazon. The norm was the cool time of the ice ages, stressed at intervals by the warmth of the interglacials. The jury is still out on the possibility of episodes of partial aridity. Throughout all these climatic changes, the forest shuffles its species over the Amazon lowlands. □

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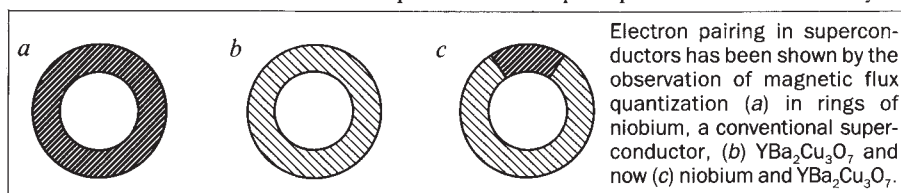
Pairs transported in phase

David Caplin

MAGNETIC-FLUX quantization in a composite ring made of a conventional superconductor and a high-temperature superconductor shows, according to Keene, Jackson and Gough on page 210 of this issue¹, that the superconducting pairs in the two materials are likely to have the same symmetry. This could be important in constraining theories of the mechanism of superconductivity in the new materials.

Three years on from the discovery of high-temperature superconductors, physicists are little nearer understanding the mechanism that makes them super-

conducting without dissipation from one kind of superconductor to the other. Also, when illuminated with microwave radiation of frequency ν , the voltage–current characteristic develops steps at voltage intervals given by the Josephson relationship $h\nu = 2eV$ (h is Planck's constant, e , the electron charge; the factor 2 reflects the double charge of the pairs). These phenomena depend on the phase coherence of the superconducting wavefunction across the junction between the two materials, so although they do not prove that the pair species are identical, they do



conducting. The behaviour of the new materials is very much like that of the conventional superconductors, which was first explained satisfactorily by Bardeen, Cooper and Schrieffer (BCS) in 1957, and it is accepted that electron pairs (Cooper pairs) are the current carriers in both species. On the other hand, the interaction that holds the pairs together is almost certainly different². The BCS electron–phonon–electron interaction of classical superconductivity seems to be too weak to account for the high transition temperature of the new materials.

The interaction can affect also the form of the pairs: in classical superconductors, the electrons have opposite spins and wave vectors, giving zero net spin and angular momentum (*s*-wave pairing). In contrast, the pairs in the superfluid phases of ³He have their nuclear spins aligned (*p*-wave pairing). Even before the advent of the ceramic superconductors, the example of *p*-wave pairing in ³He gave rise to suggestions that in some rather unusual, 'heavy fermion', low-temperature superconductors, such as UBe_{13} , the pairing is not *s*-wave³, although the inference has been disputed⁴. Some of the interaction mechanisms that are being canvassed for the high-temperature superconductors predict non-*s*-wave pairs, but the experiment by Keene *et al.*¹ adds to the evidence that the pairs in the new materials are *s*-wave, like those in the old.

How can one check on the pair species? An important clue comes from the behaviour of junctions between old and new materials. Josephson phenomena have been observed at these, just as at junctions between classical superconductors: a small but finite current can pass

show that pairs are transmitted without disrupting phase coherence.

Phase coherence and pairing can be demonstrated also by the magnetic response of macroscopic superconducting rings (see figure). Magnetic fields cause the phase of the wavefunction to rotate, so that in a ring flux has to be quantized in units of $h/2e$ (the flux quantum Φ_0). Flux quantization in conventional superconductors was first shown by Deaver and Fairbank⁵ in 1961. A few weeks after the discovery of $\text{YBa}_2\text{Cu}_3\text{O}_7$, Gough and colleagues demonstrated⁶ that in that material, the flux quantum is Φ_0 also. Now, in a variation of that original experiment, Keene *et al.* show that flux is quantized in a ring that is part conventional superconductor (niobium) and part high-temperature material ($\text{YBa}_2\text{Cu}_3\text{O}_7$) (c in the figure), and that the unit is again Φ_0 .

The experiment reinforces the previous results from single junctions^{7,9}. It certainly constrains the theorist whose pairing mechanism produces *p*- or *d*-wave pairs, for the interconversion of pair species at the boundaries would have to occur in some phase coherent manner. In some ways it is rather surprising that there is so little hindrance to pairs crossing from one

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kind of material to another, given the poor behaviour of intergrain contacts within $\text{YBa}_2\text{Cu}_3\text{O}_7$ itself, which is proving such an obstacle to the technological application of the new materials¹⁰.

All in all, the evidence from these experiments, done with relatively unsophisticated equipment (which is another way of saying that several other groups could have done the experiment

had they had the idea), is now rather strongly in favour of *s*-wave pairing in the new materials. We still await a convincing mechanism for the mechanism that holds the pairs together. □

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BACTERIA-YEAST CONJUGATION

Generic trans-kingdom sex?

Scott E. Stachel and Patricia C. Zambryski

As any student of mythology can attest, conjugal matings between disparate species will bring forth wonderful and novel creatures. Nature has wisely set barriers to such horizontal gene transfer in eukaryotes, but trans-species sex by the process of conjugation is common among prokaryotes¹⁻³. Although interspecies mating is forbidden for eukaryotes and ubiquitous for prokaryotes, biologists have largely ignored the possibility of trans-kingdom DNA transfer between bacteria and eukaryotes. To this end, Heinemann and Sprague on page 205 of this issue⁴ report the novel observation that *Escherichia coli* can conjugally transfer DNA to the yeast *Saccharomyces cerevisiae*.

During conjugation between prokaryotes, a linear single-stranded (ss) DNA is generated, transferred from a donor to a recipient cell, and regenerated into a double-stranded (ds) DNA molecule. These events are mediated by three sets of functions: *oriT* (origin of transfer); *mob* (mobilization); and *tra* (transfer). The *mob* functions process the *cis*-acting *oriT* to generate a DNA-protein transfer complex, while *tra* functions effect donor-recipient events, including production of the conjugal pilus or analogous structure for initial recipient contact, and generation of the conjugal pore through which the transfer complex probably moves. The *mob* functions are *oriT*-specific, whereas *tra* functions are often promiscuous, effecting transfer of various transfer complexes.

Heinemann and Sprague used *E. coli* donor cells harbouring a wide host-range IncPl R-plasmid carrying its cognate *tra* and *mob* functions, together with ColEI *mob* functions; and a second plasmid carrying the ColEI *oriT*, a yeast plasmid origin of replication, and the yeast *leu2* gene as selectable marker. Remarkably, they obtained *leu2*⁺ progeny yeast cells after coinoculation of *E. coli* donors with *leu*⁻ yeast recipients on solid media. This gene transfer was dependent on cell-cell contact, donor cell viability, residence of the transmissible DNA in donor cells at the time of cell-cell contact and, most critically, on the ColEI *oriT* and *mob* functions. Thus, by physical and genetic

criteria, the *leu*⁺ progeny result from the conjugal transmission of plasmid DNA from bacteria to yeast (although the structure of the transferred DNA remains to be analysed). Additional experiments demonstrated that F-factor conjugal functions can also effect bacteria-yeast conjugation.

Bacterial-yeast conjugation is not the only example of prokaryote-eukaryote sex. Nature has exploited this phenomenon for the directed transfer of DNA from the phytopathogen *Agrobacterium*

transfer complex, and the T-DNA border sequences and *vir* functions are, respectively, equivalent to *oriT* and *mob/tra* functions. Additional support for this proposal comes from genetic studies showing that the *oriT* of a conjugative plasmid will substitute for T-DNA borders to direct T-DNA transfer, as long as both *vir* functions and conjugative *mob* functions are provided in *trans*⁷. Presumably, the *oriT* and its cognate *mob* proteins generate a conjugative intermediate that moves from *Agrobacterium* to plant cells by *vir*-specified transfer machinery. Thus, bacteria conjugally mobilize DNA to at least three distinct recipients — bacteria, yeast and plant cells. We compare the functional requirements of these processes in the table.

The demonstration of bacteria-yeast conjugation by Heinemann and Sprague⁴ leads to a generalization of the phenomenon of trans-kingdom sex first observed for T-DNA transfer. This generalization should stimulate new research, including mechanistic analysis of the process itself and an investigation of its biological significance. With respect to the process, conjugation was not originally designed for eukaryotic recipients, and such reci-

Functional and genetic requirements for disparate conjugal recipients

	Bacteria→bacteria	Bacteria→plant	Bacteria→yeast
Cell-cell events			
Initial recipient contact	<i>tra</i> (pilus)	<i>chv</i> , <i>vir</i> ?	<i>tra</i> ?
Activation	mating signal; <i>tra</i>	phenolics; <i>virA/virG</i>	?; <i>tra</i> ?
Conjugal pore	<i>tra</i>	<i>virB</i> ?	<i>tra</i> ?
Transfer complex			
Transfer origin	<i>oriT</i>	T-DNA border	<i>oriT</i> (ColEI)
Nickase/pilot protein	<i>mob</i>	<i>virD1</i> , <i>virD2</i>	<i>mob</i> (ColEI)
Ssb protein	<i>tra</i>	<i>virE</i>	<i>tra</i>
Recipient events			
Primase	<i>tra</i>	<i>vir</i> ?	<i>tra</i> ?
Nuclear localization	NA	<i>virD2</i> ?	<i>mob</i> (ColEI)?
Recipient stabilization	<i>oriV</i>	chromosome integration	yeast plasmid <i>oriV</i>
Transconjugant selection	drug resistance	neoplastic growth	leucine prototrophy

tumefaciens into plant cells (reviewed in ref. 5). During this cell-cell interaction a specific element, the T-DNA, is mobilized from a donor bacterium to a wounded plant cell, localized to the plant cell nucleus and stabilized by chromosomal integration. Expression of T-DNA genes in the plant cell leads to its transformation into a crown-gall tumour which provides *Agrobacterium* with specialized nutrients. The T-DNA element is delimited by *cis*-essential T-DNA border sequences, and its transfer is mediated by the *vir* (virulence) functions. Activation of *vir* results in the generation of a unipolar ss T-DNA copy, the T-strand⁶, complexed with several proteins. The structure and mode of synthesis of this T-DNA transfer intermediate, together with the requirement for direct bacterial-plant cell contact, suggest that the T-DNA is transferred to plant cells by a mechanism analogous to bacterial conjugation. Thus, the T-strand complex is equivalent to the conjugal

ents necessitate additional cell-cell and recipient events. *Agrobacterium* clearly has mastered these events, as close to 100 per cent of surviving plant recipients can be T-DNA transconjugants. Conversely, at best only 5×10^{-5} yeast transconjugants per recipient are obtained. This frequency is still remarkable, as the bacterial conjugation systems used by Heinemann and Sprague have somehow overcome the additional complexities of conjugation to yeast. For example, the thick yeast cell wall must be bypassed to allow productive cell-cell contact. This might occur during a discrete stage of the cell cycle; for instance, membrane proteins or lipopolysaccharides that could potentially substitute for the sites used by bacteria for establishment of stable mating pairs might become exposed at the mother-daughter bud scar. Heinemann and Sprague report that the yeast transconjugant frequency measured both per recipient and per donor dramatically increases when the

mating ratio of recipient to donor is decreased. Two effects together can explain these results: bacteria are toxic to yeast, and only a few yeast cells are competent for mating, perhaps reflecting a dependence on a particular stage of the yeast cell cycle. Analysis of resistant and hypersensitive conjugal yeast mutants, as well as cell-cycle mutants, should provide insight into these cell-cell events.

With respect to additional recipient events, the conjugal DNA must find the cell nucleus, and subsequently be stabilized as a ds molecule. The transfer complex itself might in part mediate these events. The 5' terminus of the linear ss DNA, for example, is closely associated with a *mob*-encoded 'pilot' protein, which initiates the generation of the conjugal DNA and possibly mediates its mobilization through the conjugal pore, together with its protection and conversion into a stable ds DNA. In prokaryote-eukaryote conjugation, this protein might also effect nuclear localization. The *Agrobacterium* *virD2* protein, which is essential for T-strand generation, is linked through its amino-terminal portion to the T-strand. Intriguingly, the *virD2* carboxy terminus, which is dispensable for T-strand synthesis but essential for virulence, contains sequences which closely resemble nuclear transport signals of eukaryotic nuclear proteins⁸. It will be interesting to see if pilot proteins functional in bacterial-yeast conjugation also fortuitously carry karyophilic sequences, and whether these sequences can direct nuclear localization. The conjugal DNA is also typically coated by a ss DNA-binding protein, which could help in protection of the transferred DNA, and can be associated with a primase activity that might mediate regeneration to a ds molecule. Recipient-cell functions probably also help in these events; analysis of yeast DNA metabolism mutants should be instructive.

Bacteria-yeast conjugation, like the recent report of uptake and transmission of exogenous DNA by sperm⁹, is an unexpected laboratory phenomenon of horizontal gene transfer. At present, both these processes are of uncertain biological significance — do they exist in nature, and are they involved in speciation? Bacterial-yeast conjugation could occur in nature: *Saccharomyces* species are found in microenvironments (including decaying vegetation, ripening fruit) that harbour many bacterial species that carry conjugal plasmids. If such matings do fortuitously occur, they will typically be nonproductive as the transferred DNA will lack sequences for stabilization and selective expression in the transconjugant. It is also possible that specialized conjugation systems directed towards yeast recipients exist (analogous to T-DNA transfer for plant cells). With the generalization of trans-kingdom conjugation one must begin

High-temperature superconductor lens



SMALL rings of the new high-temperature superconductors could soon be found in electron-beam experiments if physicists take up a new application proposed by Hidenori Matsuzawa and colleagues from Yamanashi University. These workers have shown that relativistic electron beams are squeezed to a focus, as shown above, when their magnetic fields interact with the cooled materials.

Reporting in the *Japanese Journal of Applied Physics* (28, L717-L719; 1989), the authors recall that magnetic fields cannot penetrate superconducting samples: this is the property responsible for the 'Meissner' effect and for publicity pictures of floating magnets. The electron lens they devise consists of a passive, hollow cylinder of bismuth- or yttrium-based superconductor. As the electron beam passes down the axis of the cylinder, the magnetic field that surrounds it becomes squeezed and reacts back on the beam to focus it.

In the picture, the focused beam 2.6 mm across can be identified emerging from the left, because the authors left traces of neon gas (0.22 torr pressure) in the vacuum chamber, which is excited to fluoresce. The lens has the curious property of being more effective for more intense beams, an upper limit being set by the critical magnetic field at which superconductivity breaks down. It is not clear that the aberrations of these lenses are small enough to produce high-quality foci, but they may well be useful in applications of intense relativistic electron beams such as gas-laser excitation for which a high current density is a virtue.

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to consider whether animal cells can also serve as recipients. Intriguingly, pathogenic bacteria that express pili or adhesins for specific adhesion with epithelial cell surfaces also often harbour conjugal plasmids. Could these pathogens act as conjugal donors for the cells they infect? If so, trans-kingdom conjugation could provide a general method for producing animal-cell transformants, as it now can for plant and yeast cells. Such experiments, if possible, will necessitate strict experimental precautions.

Trans-kingdom sex might also have evolutionary significance. Several members of the plant genus *Nicotiana* carry a copy of a T-DNA gene whose expression can alter plant development; thus, an ancestral T-DNA transfer event could have contributed to *Nicotiana* speciation¹⁰. In general, however, the significance of trans-kingdom conjugation for speciation is probably minimal as fortuitous transfers will be neutral. Another possibility is that trans-kingdom conjugation allows bacteria to serve as vectors for the horizontal transmission of genes between eukaryotes. This is not a totally far-fetched idea as gene transfer from eukaryotes to prokaryotes has been reported (in ref. 11, for example). Such a

process could help explain some phylogenetic discrepancies¹².

Trans-kingdom conjugation, unlike interspecies mythological matings, will give rise to neither minotaurs nor satyrs. Nevertheless, it might mediate a low level of horizontal gene transfer in eukaryotes. It remains for future experiments to determine whether bacteria can also mate with animal cells, and to deduce what roles such disparate sex might play in nature. □

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Antarctic astronomy in the clear

SCIENCE at the South Pole has been conducted by a few sturdy individuals since the 1940s. In cosmic ray studies and astronomy, Martin Pomerantz was one of the first of these pioneers, so it was appropriate that the Bartol Institute, from which Pomerantz recently retired as director, was the host for a recent meeting* on what should be done to further astronomy at the South Pole. The meeting brought out many reasons, some obvious and some subtle, for battling the physical hardships and logistical difficulties of the Antarctic to make novel measurements.

For astronomers, the air over the South Pole has two special attributes which give it a unique transparency: the freezing cold which reduces the humidity to as little as 2 per cent, and the less well-known fact that the South Pole is more than 2,000 m above sea level, about the same as the Kitt Peak Observatory in Arizona. Together, these give the Antarctic atmosphere a transmission factor of 80 per cent in the millimetre range, where Mauna Kea in Hawaii, at close to 5,000 m altitude, can manage only 20–40 per cent (F. Lo, Univ. Illinois).

Practical submillimetre astronomy, normally done from above the atmosphere, can be done from the South Pole, where the ground base offers the hope of building telescopes of several metres in diameter; the ASTRO project (Antarctic Submillimetre Telescope and Remote Observatory), a cooperation between AT&T Bell Labs, Boston University and the University of Illinois, is a metre-class telescope designed to do far-infrared astronomy throughout the Antarctic winter (A. Stark, AT&T). To be worth while, ASTRO must eventually offer an instrument much larger than the 1-m telescope aboard the Kuiper airborne observatory, a converted Boeing 747.

The opportunities offered by this kind of astronomy, especially now that high-efficiency CCD (charge-coupled device) detectors are available for infrared measurements, are wide-ranging. Infrared observations generally pick out cool, tenuous objects such as molecular clouds, and gas and dust being blown off by old stars or coming together as new ones. The large-scale, slow and complex astrophysical processes of galactic evolution show through more clearly in the infrared (R. Genzel, Max Planck Inst.; J. Peterson, Princeton Univ.).

At the other extreme, the transient and often controversial phenomena claimed by astronomers for whom 10^{11} eV is the low end of their spectrum may best be studied from the South Pole. The advantage here lies not in any intrinsic property

of the atmosphere, but in the fact that it stays in one place relative to the object observed (T. Weekes, Smithsonian Inst.).

In very high energy astronomy (10^{11} – 10^{15} eV), an array of photodetectors on the ground is co-ordinated to look for a characteristic cone of Cerenkov radiation initiated by an energetic electron in the upper atmosphere; in ultra-high energy astronomy (10^{15} eV and above), particle detectors look for an 'air shower' of particles cascading from an initial collision between an energetic cosmic ray and an atmospheric atom. In both cases, the atmosphere is the detecting medium and the special value of observations at the South Pole is that a circumpolar celestial object is always seen through the same volume of atmosphere. The few real claims in this difficult branch of astronomy, such as the detection of periodic emission from known binary objects such as Cyg X-1, Her X-1 and some millisecond pulsars, are made less certain because the size and geometry of the region through which the instrument is looking changes with the Earth's rotation.

SPASE (South Pole Air Shower Experiment), an array of 14 scintillator detectors 30 m apart and situated 200 m from the South Pole (A. Hillas, Leeds Univ.) has recorded air showers throughout a southern winter. A bigger version is planned, but already it has demonstrated the feasibility of running a full-time experiment through the months of winter darkness.

More extravagantly, T. Stanev (Bartol Inst.) mentioned two versions of an instrument which uses ice, not air, as the detecting medium. In one, photomultiplier tubes would be equipped with heaters and allowed to fall several kilometres through the ice to rest in what is thought to be an expanse of pure, clear and bubble-free ice. There they would form a vast Cerenkov neutrino detector, like a bigger and frozen version of the Kamioka or IMB water-Cerenkov detectors that found the neutrinos from SN1987A. In a variation on this, Stanev described an idea due to the Russians Markov and Zhelezniak for making a radio-Cerenkov detector in the ice. Neutrino-initiated electron-positron showers develop a charge asymmetry as they propagate; in ice they have a cross-section measured in metres. This moving small-scale charge emits radio waves, which could in principle be detected by microwave horns on the surface of the ice. Some experiments have been done at the Russian Vostok base, but the ice appears to be noisier than expected, and may prohibit a realistic detector of this sort.

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Personal growth

A SUBTLE debate has been convulsing a sub-section of the feminist movement. Is it politically correct to use disposable babies' nappies, thus minimizing women's degrading unpaid household labour? Or are re-usable towelling nappies, saving the environment, ideologically more virtuous? Daedalus points out that many primitive peoples avoid this dilemma by using sphagnum moss as an absorbent for infantile excreta. The discarded moss re-enters the natural cycle, and may even survive and benefit from its fertilizing experience.

So Daedalus is devising the ultimate nappy. Drawing heavily on mustard-and-cress flannel technology, it consists of an inert textile backing carrying a heavy growth of moss. After use it is simply laid aside; the fertilized moss soon metabolizes the excreta, producing more moss. It can then be used again. This cunning product solves the whole problem. It even grows most copiously in those areas most exposed to use, so that it shapes itself to its wearer's exact needs. A small initial supply of such truly 'green' nappies can be used and re-used endlessly.

DREADCO textile technicians are now seeking to broaden this elegant principle. Some adult clothes (like sweat-bands and bandages) also exist primarily to absorb biological exudates. And all clothes get dirty, on the outside from environmental grime and on the inside from human perspiration and skin detritus. Living clothes which absorb dirt and build it into new fabric would brilliantly solve the twin nuisances of laundry and repair.

So DREADCO's biologists are seeding synthetic base-fabrics with various micro-fibrous plants to create a sort of biological corduroy. They are still debating whether small grasses, moss or algal filaments will be best, or whether the cloth can be constructed as a stable ecosystem of several mutually dependent species. Either way, the new living fabrics seem set to transform the fashion scene.

Self-cleaning, self-repairing and ecologically virtuous, 'Livewear'® will even keep its wearer clean by continuous gentle dermal scavenging. It will come in rather muted mossy and lichen-like colours, whose variegated pattern will slowly grow and change, especially at underarms, knees, elbows, and other moist or dirt-trapping areas.

Livewear will also exercise a useful discipline on fashion fanatics. Regularly worn, a Livewear garment will be moistened and fertilized by its owner; wear and tear will be renewed and it will last indefinitely. But unworn, languishing in a dark, dry wardrobe, it will soon wilt and die. Surplus garments will automatically weed themselves out, while the timeless favourites live on and flourish.

David Jones

* *Astrophysics in Antarctica*, Bartol Institute, University of Delaware, 8–10 June 1989.

Ubiquitin and dementia

SIR—Gallo and Anderton¹ draw attention to the demonstration by ubiquitin immunocytochemistry of ubiquitin-immunoreactive intraneuronal inclusions in the major neurodegenerative disorders, including Alzheimer's, Parkinson's and motor neuron diseases.

We wish to make two additional points. First, we believe that diffuse Lewy body disease can be added to Gallo and Anderton's table of common neurological diseases characterized by the presence of ubiquitinated filamentous inclusion bodies. We and others have recently shown that ubiquitin immunocytochemistry, coupled with classical neuropathological techniques, reveals diffuse Lewy body disease as a common cause of dementia^{2,4} — it accounts for 15–27 per cent of cases of dementia in hospital necropsies, whereas it was previously considered rare. We have also used ubiquitin immunocytochemistry to study the disorder quantitatively, and have shown that severity of dementia is related to the density of cortical inclusions but not to measures of sub-cortical pathology⁵.

Second, ubiquitinated filamentous inclusions are not confined to neurons but also occur in astrocytomas and, outside the nervous system, in alcoholic liver disease, a myopathy⁶ and in some viral diseases⁷. Many (but not all) of these inclusions contain intermediate filaments^{1,6}. The significance of this last observation could relate to the cell stress or 'heat-shock' response. It is well known that

experimental chemical and thermal stresses cause specific, reversible intermediate-filament collapse in various cell types⁸. We have recently shown⁹ that the expression of a human polyubiquitin gene is increased in spinal cord and brain in motor neuron disease. Polyubiquitin genes are inducible by heat shock and are essential for cell survival¹⁰.

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Silica polymorphs

SIR—In the contents panel of *Nature* of 18 May 1989, an explanation of a paper¹ on zeolite is accompanied by a figure that is, in fact, taken from our paper² in the same issue on new crystal structures of silica (SiO₂). Curiously, this inadvertent association reminds us of a notion that has

been at the back of our minds for some time — namely, that silica, which has typical framework structures comprising networks of tightly bound units (SiO₄ tetrahedra in this case), could assume open crystal structures that are reminiscent of zeolites.

As an old example³, melanophlogite, which is mentioned in a standard textbook

MIRAGE or no mirage?

SIR—Brenner *et al.*¹ doubt the potential of the Mössbauer-isotope-based method of cancer therapy we described² because of their calculation that several greys of low-energy gamma rays would be necessary to achieve the same effect by conventional mechanisms that we achieved with 10⁻⁵ Gy of resonant Mössbauer radiation. There are three reasons for this incongruity. First, although the cross-sections are small for all atoms found in tissue, so that most of the gamma rays are absorbed by water molecules and produce random transient free radicals, the Mössbauer cross-section for ⁵⁷Fe (with resonant 14.4-keV gamma rays) is about 100,000 times larger.

Considering the abundance of water molecules and ⁵⁷Fe atoms in these cells, and considering the cross-sections of water and ⁵⁷Fe, we estimate that about half of the gamma photons will be absorbed by ⁵⁷Fe atoms. Second, the strategic position of the ⁵⁷Fe atoms (bonded to molecules that are intercalated in the DNA), makes absorption by ⁵⁷Fe atoms much more effective than absorption by random water molecules. Third, gamma-photon absorption by ⁵⁷Fe produces an Auger cascade, of about eight high-LET electrons, and a highly-charged iron atom, within a femtosecond. Based on our results and on the results of radiolabelling experiments with a similar mechanism, we estimate the relative cell-killing efficiency of our MIRAGE processes compared with the conventional radiotherapy processes at about 10⁵ to 1.

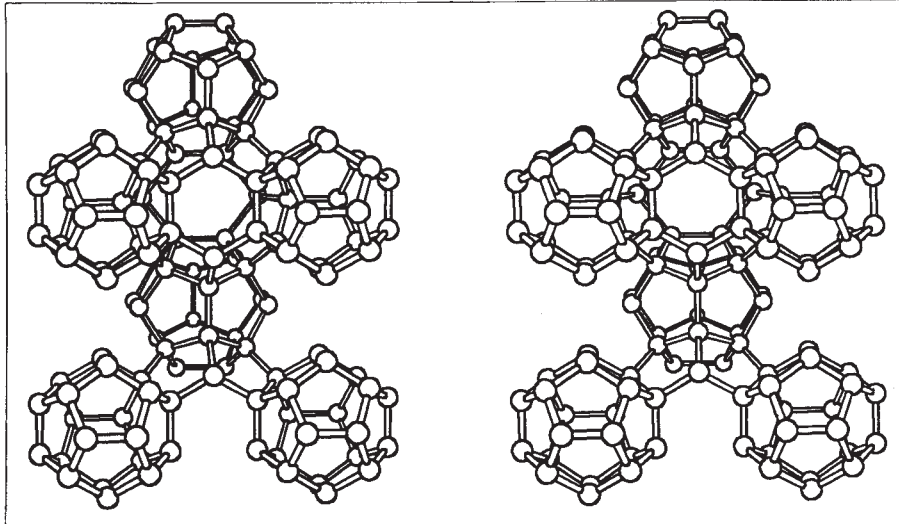
Humm³ points out that ⁵⁷Fe would only be useful for superficial lesions and has asked for a control experiment at a Doppler shift well off the Mössbauer peak. In response to the former, we observe that it would be possible to use Mössbauer isotopes with more penetrating gamma

by Wells⁴, is thought to have a silicon framework of chlorine hydrate structure⁵, as shown in the figure. Although this particular polymorph could be an impure phase of silica containing some elements such as sulphur or carbon, it serves to indicate that silica and related compounds could assume more polymorphs than currently expected.

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rays, such as ^{119}Sn , ^{151}Eu or ^{121}Sb . As for the control, in the early stages of our work we carried out one experiment with a source velocity of 0 mm s^{-1} and observed a decrease in cell proliferation (compared with the 'drug alone' group) but much less of a decrease than observed with the Doppler shift on the Mössbauer peak ($+1.5\text{ mm s}^{-1}$). We attribute this small effect to the presence of Fe(II)bleomycin , which would absorb at 0 mm s^{-1} , in the medium *in vivo*.

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Constraints on brain growth

SIR—In birds, adult brain size is larger in altricial species, which produce relatively undeveloped and dependent hatchlings, than in precocial species of similar adult size, which produce well-developed and functionally independent hatchlings¹. By contrast, the relative adult brain size of placental mammals varies independently of the developmental state of the neonate². Although this difference between birds and mammals is striking and may have far-reaching ecological and evolutionary implications³, no hypothesis has yet been offered to account for its existence. We now suggest that it is a consequence of fundamental differences in the availability of substrates for metabolic processes in the embryonic brain.

Unlike most vertebrate tissues, the central nervous system (CNS) uses carbohydrates or, in part, ketone bodies as its main catabolic substrates; the direct catabolism of lipids contributes only minimally to its energy requirements⁴. Within the confines of the avian egg, however, carbohydrates are unsuitable as an energy-storage medium because of their low energy density. Instead lipids, which have an eight-fold higher energy density than carbohydrates, account for 84–98 per cent of the substrates oxidized by the avian embryo⁵.

The limited stores of carbohydrates in an avian egg and the inability of CNS tissues to rely on the direct catabolism of lipids means that the metabolic demands of the CNS in avian embryos, particularly

in the later stages of development, must be met by glucose derived from either glycerol or amino acids and by ketone bodies generated from fatty acids. This has its costs. First, the use of ketone bodies in lieu of glucose leads to a high concentration of blood ketone bodies, which may require additional resources to buffer the associated ionic imbalances and may inhibit proper CNS developments⁶. In addition, the relatively low energy density of amino acids, comparable to that of carbohydrates, makes them of limited use as gluconeogenic precursors in eggs, and their catabolism produces nitrogenous wastes that must be sequestered from the developing embryo.

A further problem in the egg arises from the fact that the CNS is more active metabolically than most other tissues of avian embryos and has a correspondingly higher relative rate of energy demand and oxygen consumption. Constraints on the supply of oxygen across an eggshell may, therefore, limit brain growth.

Unlike embryos in an egg, avian neonates can readily obtain carbohydrates and gluconeogenic substrates either for themselves or from their parents. They also have unlimited access to oxygen and can freely excrete nitrogenous wastes.

Altricial birds are thought to have evolved from smaller-brained precocial ancestors⁷. In extant precocial species, the ontogeny of the CNS is characterized by substantial pre-hatching differentiation and myelination of neurons^{8,9}, presumably because of the behavioural and metabolic demands on precocial hatchlings. One consequence of a larger adult brain may be a parallel increase in brain size at all equivalent stages of brain ontogeny⁸. In this case, the evolutionary increase in adult brain size would have required a concomitant increase in the size of the embryonic CNS, and hence in the total metabolic costs incurred to reach a functionally comparable stage of development at hatching. The relatively high costs of supporting prenatal CNS catabolism, as outlined here, might then have favoured a decrease in the proportion of brain growth and differentiation that occurred prenatally (increased altricity) with an associated increase in parental care after hatching.

Energy use by mammalian embryos is fundamentally different from the lipid-based metabolism found in the eggs of birds and all other oviparous vertebrates. The almost exclusive catabolic substrate of mammalian fetuses is maternally supplied glucose; use of lipids is rare or absent⁶. Thus, mammalian fetuses have ready access to carbohydrates during early development, which oviparous vertebrates do not have until after hatching. For most mammals there is no extended period during development in which substrates for CNS metabolism are limited. Thus, there is relatively little difference

between the pre- and postnatal costs of supporting CNS metabolism relative to those for other organs, the prenatal ontogeny of the mammalian brain is not constrained by embryonic nutrition, and an inverse relationship between developmental stage at birth and adult brain size is neither required or observed.

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Biological light guides

SIR—Wells¹ suggests that the question of "whether light transmission down hairs affects skin and hair" needs investigation. In that respect, it is worth pointing out that many major vertebrate sensory systems incorporate cilia in their structure, including the hair cells of the cochlea, the oriented hair cells of the vestibular mechanism and the photo-receptor cells of the retina. The latter have been shown to have light-guiding properties (see ref. 2 for a review).

The invagination of the neural groove in the surface ectoderm of the embryo leads to the development and elaboration of the nervous system. The infolding and incorporation of surface tissue within the embryo accounts for the presence of ciliated cells within nervous tissue. Neurons, which after maturation form photoreceptors containing cilia, line the outer wall of the collapsed brain ventricle that will form the retina. A study of the evolution of the relationships between cilia and specific sensory systems would be of interest.

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Kissing and making up

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Peacemaking Among Primates. By Frans de Waal. *Harvard University Press*: 1989. Pp. 294. \$29.95, £23.95.

FRANS de Waal's first book, *Chimpanzee Politics* (Harper & Row, 1982)* caused a stir. It transformed a colony of chimpanzees at Arnhem Zoo in the Netherlands from the subjects of academic research almost to the status of players in a soap opera. Stunning photographs, crisp prose and insightful stories combined in a compelling saga of power and sex among the apes. Backed up by careful research published elsewhere in scientific journals, *Chimpanzee Politics* was easily accessible to the general public, making it perhaps the closest captive counterpart to Jane van Lawick-Goodall's *In the Shadow of Man* (Weidenfeld & Nicolson, 1988). Given this background, it is not surprising that expectations were high for de Waal's next effort.

Peacemaking Among Primates is in many ways more ambitious, though de Waal has sacrificed depth for breadth. He tackles not one but four species of non-human primates: in addition to chimpanzees, bonobos (or pygmy chimpanzees), rhesus macaques and stump-tailed macaques are studied. Moreover, he also seeks to compare the findings to another species of primate, *Homo sapiens*. Thus the book invites comparison with the works of other 'ethologizers' (to use Hilary Callan's term) such as Irenaüs Eibl-Eibesfeldt, Konrad Lorenz and Desmond Morris.

The author's avowed aim is "... to correct biology's bleak orientation on the human condition". This is done on the explicit assumption that the study of animal behaviour sheds light on the roots of our own society. Thus de Waal's appeal to the general reader is a prescriptive as well as a descriptive one, and this is a source of both strength and weakness in the book.

The first chapter sets out the rationale. The main theme is that scientists studying the "social fire" of aggression have concentrated on the ignition of the flames but have ignored how these flames are later extinguished. De Waal argues that group-living organisms both compete and

cooperate, so that in order to stay together they must manage the former to enjoy the latter. The necessary process is reconciliation. Relationships must be repeatedly serviced and renewed and never taken for granted, and each species handles this differently, in its own social framework.

For chimpanzees, much of the peacemaking was described in *Chimpanzee Politics*, to which the chapter in the new book is largely a sequel. Ironically, it

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Stump-tailed macaques: "ritualized conflict resolution... made all the more impressive by their striking individual differences in appearance".

details the fatal effects of failed reconciliation, when the dominant male is killed by others. As with field studies of wild chimpanzees at Gombe, there is an air of 'loss of innocence' about the emergence of deadly violence after years of apparently peaceful relations. The 12 pages over which the story unfolds are gripping.

The chapter on rhesus macaques is the least surprising given how much this south Asian species of monkey has already been studied in a multitude of settings. De Waal emphasizes the foundational role of matrilineal rank but goes beyond this to talk of social classes, and of the female community's collective support for the system. Rapprochement in rhesus monkeys is less a matter of explicit reconciliation than of implicit tension-breaking.

Stump-tailed macaques get the least

space but come across in many ways as the most interesting of the four species. Their reconciliations are often elaborately orgasmic, both homo- and heterosexually. Their conflict resolution seems highly ritualized — from mock wrist-biting to multiple embracing — and is made more impressive by their striking individual differences in appearance. A detailed field study of this species in south-east Asia is long overdue.

Bonobos continue to fascinate, though it is not yet clear whether the differences between them and chimpanzees are ones of style or substance. Curiously, work on captive bonobos lags behind that on wild ones. De Waal's is the principal behavioural research project, on the biggest colony, at San Diego Zoo. Bonobos are playful hedonists, fond of pulling faces and varied sex. Sexual relations are the currency for negotiating possession of resources or moderating aggression.

One can assess de Waal's treatment of the four species of non-human primates in terms of the three 'false dichotomies' which he raises early on in the book. The real problem is the dichotomy between field and laboratory studies, and is thus one of validity. One can never properly simulate a tropical forest in a temperate zoo, but one can try to simulate the crucial social and environmental variables. This works better for some species than for others. A community of chimpanzees in nature virtually never gets together; in captivity a group of chimpanzees is *always* together. Phenomena arising from this — such as females mediating in males' disputes (as described by de Waal at Arnhem) — have not been seen in the wild, and so although they may be suggestive, they may be artefacts of captivity.

De Waal's treatment of the fifth species of primates, ourselves, is the most frustrating. No one would doubt the importance of reconciliation to human beings at many levels — personal, familial, institutional, international. But here telling incidents and plausible speculation are not enough; anecdotal cross-cultural comparisons are provocative but not conclusive. De Waal is aware of the gaps in knowledge and bemoans them. His final chapter should be seen mostly as a consciousness raiser, serving to stimulate much-needed research on peacemaking in human primates. □

*A paperback edition of this book was published earlier this year by Johns Hopkins University Press. Price is \$12.95.

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At the centre of things

Anthony W. Thomas

Models of the Nucleon: From Quarks to Solitons. By Rajat K. Bhaduri. Addison-Wesley: 1988. Pp. 360. \$38.95, £34.95.

IT is no minor task that the author attempts here, namely to present a text-book summary of the various models of nucleon structure, including their experimental basis, for the nuclear physicist. Given the intended audience, quantum field theory is not used; and for the same reason there is an introductory account of gauge theories (including the Weinberg-Salam model and quantum chromodynamics).

Perhaps because of the breadth of the task which Bhaduri has set for himself, the quality of the treatment varies enormously. The discussion of deep-inelastic scattering is rather weak and is effectively disconnected from the rest of the book. On the other hand, the non-relativistic quark model and the MIT bag model are well treated. There is a digression into the difference between Dirac and Majorana neutrinos, which seems a little out of place. In the thorough discussion of chiral symmetry, the σ -model (which is a beautiful textbook example) is endowed with rather too much physical significance. The large- N_c expansion is also given too much prominence for my taste, and Witten's conclusion that the nucleon might be a soliton built from weakly interacting meson fields is uncritically repeated.

Some of the nucleon models, particularly the Skyrme model and the ideas of topological solitons, are very clearly explained. This alone will make the book valuable for graduate students working in strong-interaction physics. But one cannot help feeling, as Bhaduri himself suggests, that "it is too early to write a text on these rapidly developing areas". The author has an understandable wish to draw conclusions before it is strictly possible to do so; for example, the point is never made that the main assumption underlying all the topological soliton models is that the effective scalar field confining the quarks is the chiral partner of the pion. This may or may not be true in nature, but to present such models as the apex towards which the whole book is directed is, in my view, misguided.

Nonetheless, this is an impressive piece of work. If read with appropriate caution, it will be prove to be a useful reference volume. □

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Internalizing

Pere Alberch

Morphogenesis and Evolution. By Keith Stewart Thomson. Oxford University Press: 1988. Pp.154. £24, \$29.95.

UNTIL the early 1970s evolutionary theory was dominated by neo-darwinism, central to which is the idea of adaptation. Organisms evolve by adapting to changes in selective pressures, while at the same time morphological novelties are introduced by genetic mutation. The darwinian view stressed the deterministic properties of natural selection, taking little notice of the problem of how new variation is introduced into a population; that is, how much intrinsic order exists before the action of natural selection.

Data from palaeontology and biochemical systematics now suggest that genetic and developmental regulation may be important sources of novelties in evolution. In consequence, the integration of development and evolution is widely perceived as one of the most exciting and potentially productive 'new' areas of evolutionary biology. As Thomson points out in the book under review, "if we are to progress in evolutionary biology beyond the study only of the contingent, and of unique empirical events, we will need a general theory, and part of that theory must derive from theories of the developmental processes that drive the introduction of variation" (p.69).

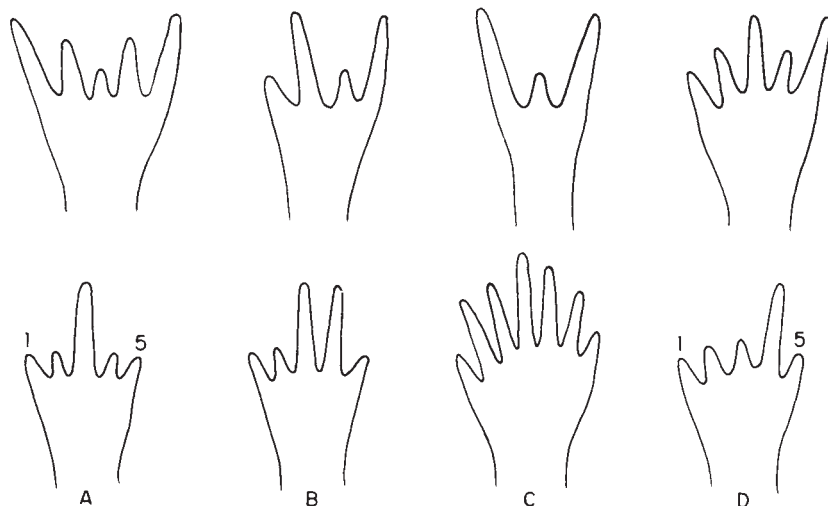
The basic idea is that developmental systems are endowed with intrinsic regulatory and pattern-generating properties. The genetic, biochemical, cellular and tissue interactions that characterize developmental systems constrain the realm of possible morphologies, in the

sense that even if the source of variation — genetic mutation — is random, its phenotypic expression is not. Certain morphological transformations are more likely to occur than others.

Two corollaries follow. First, the order that we observe in nature results from the interplay of two deterministic factors: internally generated developmental constraints and externally imposed natural selection. Second, if we were to have a good enough knowledge of a specific developmental system we could predict which kind of evolutionary transformation is more likely to occur. Together, constraints on the introduction of phenotypic variation, the limits of selection and predictability in evolutionary processes prescribe a qualitatively new research programme in evolutionary biology, one that concentrates more on the internal organization of the system and less on adaptation.

In recent research on development and evolution, the emphasis has been on mechanisms of morphogenesis, gene regulation and pattern formation rather than on the more traditional approaches based on comparative embryology. Thomson's book emphasizes mechanism over pattern. As the author himself says in the introduction, "the book has nothing to do with the old subject of recapitulation". Instead he concentrates on the role that morphogenetic mechanisms play in evolutionary processes.

Some books in science are useful because they provide a comprehensive review of a specific area, bringing together material from different sources into a unified and clearer context. In others — and such books are to be valued even more highly — the author advocates a particular, and novel, thesis. Leo Buss's *The Evolution of Individuality* (Princeton University Press, 1988) is a recent example:



The strong, single axis of orientation in tetrapod limbs means that medial digits persist in evolution, at the expense of lateral ones. The top row shows "forbidden" morphologies; the bottom, "allowed" morphologies.

From *Morphogenesis and Evolution*

not everyone will agree with the author's basic tenets, but the result is undeniably thought provoking. *Morphogenesis and Evolution* does not fall into either of these categories. It is not a comprehensive review (nor does the author intend that it should be) but neither does the book propose a qualitatively new theory of development and evolution.

I question some of Thomson's choices of references, his areas of inquiry, and his omission of relevant research now being done in developmental biology. For example, I fail to see the reason for including Chapter 5, on early pattern formation in amphibians, while at the same time ignoring the work on cell-adhesion molecules by Edelman and colleagues, work that is specifically related to morphological evolution. Likewise, Thomson's decision not to enter into aspects of genetic regulation of development is a real drawback of the book. I agree with him that "there are fundamental questions concerning evolutionary mechanisms that can only be asked and answered at the morphogenetic level" (p.vi). But I would also argue that gene action cannot be

dissociated from developmental processes; gene expression is both the cause and the effect of a series of interactions at the cellular and tissue level.

The best parts of the book are those dealing with the conceptual framework of the issues — Chapter 2, "Theory, Reduction and Hierarchy", and Chapter 3, "Development: Pattern and Process", are well written and are useful introductions to their subjects. Chapter 8, "Patterns of Evolution", is also good. Indeed, as a chronicle of "a subject in transition, a subject that is still defining itself" (p.v), the book is a success. But Thomson proposes his particular definition of the subject, and in my view his definition is incomplete because two aspects of it are ignored: the role of genes within developmental systems, and the mathematical properties of pattern-formation models. I believe that both of these topics will have a prominent part to play in formulating a comprehensive theory of development and evolution. □

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On the surface

H. K. Christenson

Colloidal Systems and Interfaces. By Sydney Ross and Ian D. Morrison. Wiley: 1988. Pp.422. £47.50, \$49.95.

ONE of the more interesting problems in applied colloid science is that of pondering over why beer foam is more stable than champagne foam. If observation and experiment get you nowhere, the answer can be found in this introductory text, which is based on a short course on emulsions and dispersions given by the authors and F. M. Fowkes.

As might be expected, the treatment of emulsions and dispersions is more detailed than is usual in this sort of book, but overall Ross and Morrison have succeeded in providing a fairly extensive overview of 'wet' surface chemistry and colloid science. Although clearly directed at an industrial audience, the book is well-suited for a more general readership. It is easy to read and important phenomena are illustrated with historical anecdotes and practical examples. Particularly valuable are the extensive bibliography and the reference lists, which are not often found in elementary textbooks. The practical sections on measurements of surface tension, particle sizing and various other techniques should be invaluable to those requiring a quick and comprehensive overview.

There are a few noteworthy omissions, no doubt stemming from the perceived

interests of the particular group for which the book is intended. In particular, there is virtually nothing on biological colloids. Some of the material on surface forces is not quite up to date, with hardly a mention of solvation and hydration forces and nothing on hydrophobic interactions. Similarly, the section on amphiphilic aggregation structures, while welcome because many textbooks on colloid and surface science contain nothing whatsoever on this topic, might well have included a brief treatment of geometric packing constraints.

There are a few other problems. The book is not well organized, many concepts being introduced before they are properly defined and discussed. It is also marred by a few sweeping generalizations and erroneous statements. Thus, potential energy is often used when free energy is meant; all liquid crystals do not flow like liquids; and retardation of Van der Waals forces cannot always be ignored in lipid-water systems. Many figures are inadequately captioned and are almost impossible for the uninitiated to interpret.

In spite of these shortcomings (easily rectified in a future edition) the book can be recommended to anyone who desires an accessible and interesting introduction to colloid science. With a slight reorganization and some additional material, it would be extremely suitable for courses at an elementary level. □

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PROTEIN-NUCLEIC ACID INTERACTION

Volume 10 Topics in Molecular and Structural Biology

*Edited by W. Saenger and
U. Heinemann.*

The book contains articles highlighting the rapidly evolving field of protein-nucleic acid interactions. Individual chapters demonstrate the diversity of biological systems to which protein-nucleic recognition is of central importance.

There are chapters on the interaction of small proteins with DNA and RNA as well as on chromatin organisation. Care has been taken to include accounts of many different experimental approaches to studying the subject. All articles are written by scientists actively involved in the research they describe and considered experts in their respective research areas. The book is well-illustrated and contains extensive bibliographic information to provide easy access to the original literature.

Contents DNA-Protein Interactions in the Regulation of Gene Expression - P.H. von Hippel (University of Oregon, Eugene, USA) and O.G. Berg (Uppsala University, Sweden). / Structures of Protein-Nucleic Acid Complexes in Solution by Electro-optical Analysis - D. Porschke and J. Antosiewicz (Max-Planck Inst., Göttingen, FRG). / NMR Studies of Protein-DNA Recognition. The Interaction of LAC Repressor Headpiece with Repressor DNA - R. Kaptein, R. Boelens and R.M.J.N. Lamerich (University of Utrecht, Holland). / The Single-stranded DNA Binding Protein of *Escherichia coli* - J. Greipel, C. Urbanke and G. Maass (Medizinische Hochschule, Hannover, FRG). / Protein-Nucleic Acid Interaction in Tobacco Mosaic Virus - G. Stubbs (Vanderbilt University, Nashville, USA). / Structural and Functional Studies of Ribonuclease T1 - U. Heinemann and U. Hahn (Freie Universität, Berlin, FRG). / Tet Repressor-Tet Operator Interaction - W. Hillen and A. Wissmann (Inst. Mikrobiol. and Biochem., Erlangen, FRG). / Structure and Condensation of Chromatin - M.H.J. Koch (DESY, Hamburg, FRG). / Conclusion / Index

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Silicon vision

Igor Aleksander and Helen Morton

Analog VLSI and Neural Systems. By Carver Mead. Addison-Wesley: 1989. Pp.371. \$39.95, £21.95.

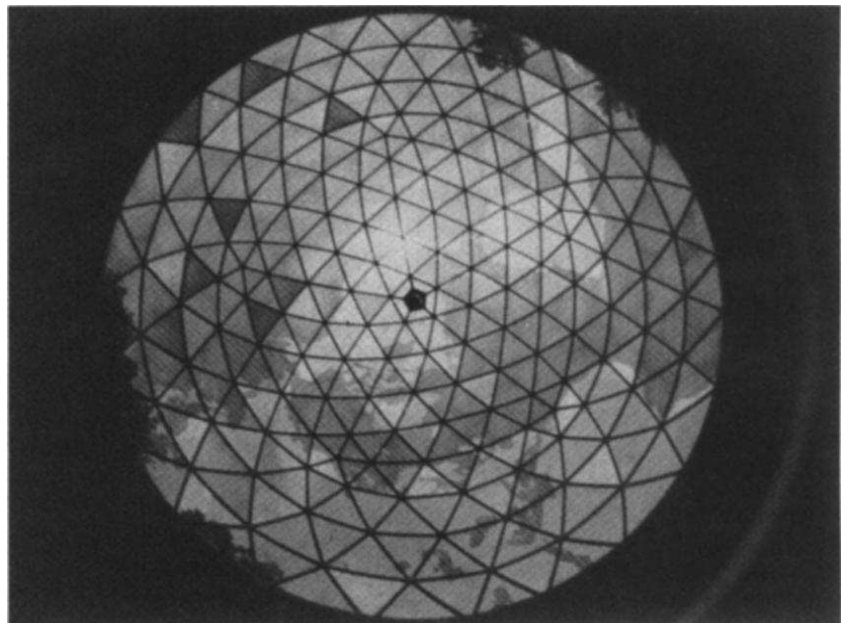
THE title, *Analog VLSI and Neural Systems*, coupled with the name of the author, Carver Mead, suggests that here is an unmissable educational opportunity for the reader. And education it will be, as the combination of Very Large Scale Integration (VLSI, or simply, the making of silicon chips) and neural systems is a topic which, as yet, has no experts.

Mead is an acknowledged innovator in silicon chip design methods, and neural systems (the study of brain-like computers) is reportedly the hottest area of research among a broad community which stretches from computer scientists and electronic engineers to neurobiologists, including physicists and statisticians along the way. Although the drawing on the cover of the book hints at the possibility that a silicon brain is now ripe for manufacture, the notion is soon tempered by Mead in the preface: "Integrated circuit fabrication has evolved to the point where systems of the scale of small, but identifiable, parts of the nervous system can be emulated on a single piece of silicon. . . . We are not limited by the constraints inherent in our fabrication technology; we are limited by the paucity of our understanding".

The book is clearly intended first to substantiate the assertion that silicon technology has developed to the right level for making devices which function like small corners of the nervous system, and second to make a dent in the "paucity of understanding". The strategy underlying the choice of material in the earlier parts becomes apparent from a glance at the last four chapters. They describe four silicon chips which Mead and his colleagues have developed, all of which are actual emulations of parts of the nervous system.

The first is a device (called SEEHEAR) which transforms a visual image into a stereo sound "tapestry" aimed at helping the blind. So, rather than emulating those nervous functions that exist in a brain, this device might be of help to those who have lost part of their perceptive capacity (sight) by providing a neural system which enhances the part that remains. Although the text does not reveal how unsighted subjects fare with this prosthesis, the idea is fascinating and draws attention to the fact that the benefit that could be obtained from using silicon in aids of this kind is truly enormous.

The second silicon system is a neural motion detector. It is known that an important function of early stages of vision in the brain is to respond to the



Looking up — Buckminster Fuller's "tool for individuals to observe Earth and the relationships between human beings and our planet". Taken from *Buckminster Fuller's Universe: An Appreciation*, by Lloyd Sieden, published by Plenum.

motion of images. Artificial systems of this kind may be essential to the development of sighted robots capable not only of reacting appropriately to moving objects, but also of controlling their own motion on the basis of visual input. The third application is simply called "a silicon retina". This not only reproduces an image received through sensors as neural firing patterns, but produces an output which is largely independent of illumination level and enhances the edges of objects in the visual image. Both of these properties are crucial in the process of 'seeing', and Mead's model is, again, a step towards sighted machines.

Having dealt with vision and the generation of sounds, the last chip, as one might expect, is an electronic cochlea, that organ of hearing that separates the frequencies of a sound into spatially distinct activity in a brain. Again the potential for helping the deaf is apparent, as is the possibility of applying the chip as a 'front end' for a voice-sensitive computing machine. Crude approximations have existed for some time but Mead's chip must be appreciated for its compactness and sophistication.

The earlier parts of the book explain the details, neural and electronic, of the ingredients of the devices featured in the last four chapters. It is an excellent survey of electronics, analogue VLSI operation, neural functions and chip fabrication techniques. Mead calls it "electronic" that is, "the common framework of electrical properties used for information processing in both brains and silicon". The combination is enticing as a subject for new curricula in both technology and neurophysiology.

One thing that the book is *not* is an

explanation of what is currently known as 'neural nets', 'connectionism' or 'parallel distributed processing'. This is the subject alluded to earlier which is being given feverish attention by so many scientists and technologists. The distinction between this and Mead's work centres on the key factor possessed by neural tissue: the ability to build up experience or acquire knowledge. Mead has not expressed a central interest in 'learning' and mentions it only in passing. To this extent he does not address the central concern of those whom one might call the neural systems engineers.

This is not a criticism: in fact, neural systems engineers well versed in connectionism would benefit from reading the book and discovering the sophisticated ways in which fixed (as opposed to 'learning') neural systems can turn sensory data of a vaguely defined nature into well-defined data which facilitate the task for neural nets which learn. The main thrust of Mead's approach is that designers intending to produce the ultimate silicon brain can hardly afford to miss any technological tricks found in the natural counterpart. As he observes, knowing about both brains and silicon need not be like attempting to build flying machines that have feathers and flap their wings. It is more a case of seeing the organizing principles of brains as a basis for silicon systems in the same spirit as a man-made glider is a sign of human appreciation of the elegance of a soaring bird. □

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Geochemical consequences of *in situ* crystallization

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In situ crystallization of a magma chamber in a transition zone between solidus and liquidus may cause the magma to evolve along a chemical path that differs markedly from that produced by fractional crystallization in closed or periodically replenished magma chambers. The chemical evolution expected from *in situ* crystallization is consistent with the data from the Kiglapait layered intrusion and may account for some enigmatic features of the chemistry of volcanic and plutonic rocks from diverse tectonic environments.

MUCH of the interpretation of geochemical data from igneous rocks has been based on the concept of fractional crystallization in magma chambers, which is often modelled quantitatively using the Rayleigh fractionation equation¹. In recent years fractional crystallization in magma chambers has been incorporated into models of 'open-system' processes that include periodic injection, eruption and assimilation²⁻⁶. Fractional crystallization is associated with the physical idea of crystal settling, which was thought to be exemplified in layered intrusions, which are exposed examples of solidified magma chambers⁷.

Over the past decade, however, new views have emerged on

how crystallization proceeds in magma chambers, with the recognition that the probable sites of solidification are the magma-chamber margins, where the heat is lost, where thermal and mechanical boundary layers exist and where there must be a transition between the (mostly liquid) chamber interior and the (entirely solid) enclosing rock. McBirney and Noyes⁸ and Campbell⁹ were among the first to re-emphasize that Jackson's¹⁰ concept of 'bottom crystallization' or *in situ* crystallization may be correct, and that solidification occurs in a transition zone containing crystals on the margins of the main, convecting body of magma (Fig. 1).

A curious consequence of *in situ* crystallization is that if liquid magma is added to the transition zone and all crystallization takes place there, in isolation from the main magma chamber, then there is no separation of crystals from the main body of magma, and hence 'crystal fractionation' does not happen. But it is clear from natural volcanic and plutonic rocks that magmatic differentiation does occur in magma chambers. The question is, how?

One possibility involves the return of residual liquid from the crystallizing margins of the magma chamber to the interior (Fig. 1a). The potential importance of such return of liquid, particularly for convective processes under certain conditions, has already been widely discussed (for example, refs 11, 12), and there is increasing evidence that such return of liquid does happen. Irvine¹³ pointed out that some of the intricacies of chemistry in the cumulate pile of layered intrusions suggested

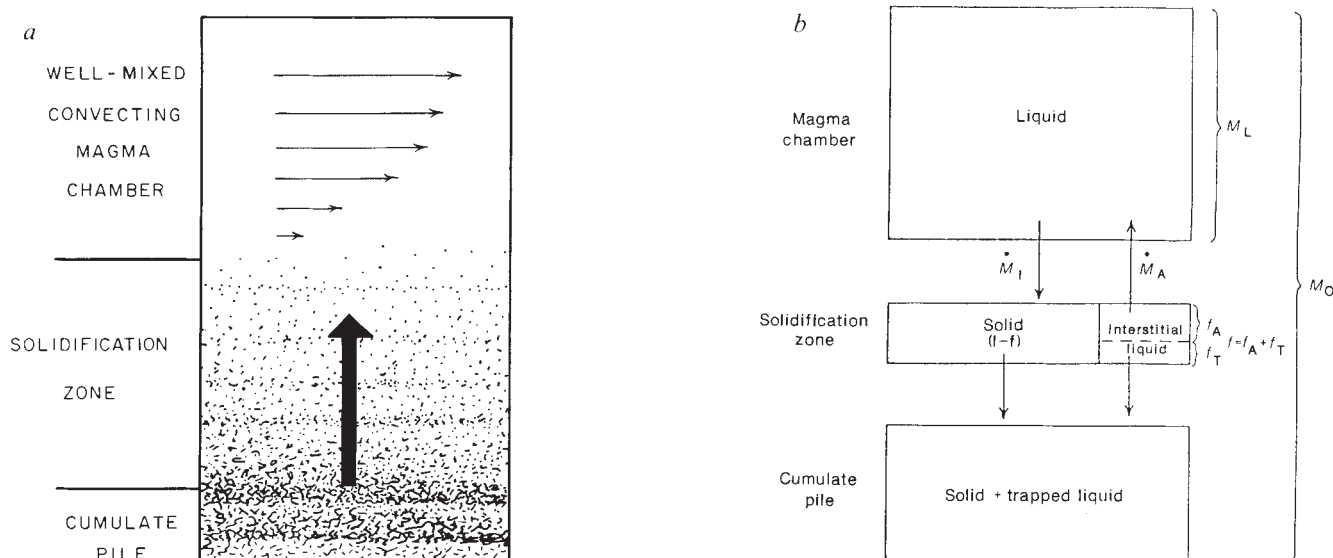


FIG. 1 a, Schematic diagram of *in situ* crystallization, modified after Jackson¹⁰ and McBirney and Noyes⁸. The vertical arrow denotes return of liquid from the partially solidified chamber margin to the interior of the magma chamber. Horizontal arrows denote convective flow in the magma chamber. Although the figure is drawn for the chamber bottom, the process could act on all sides. b, Box-model representation of *in situ* crystallization for which equations 1-6 are developed. Crystallization occurs in a solidification zone which moves progressively through the magma chamber as solidification proceeds. Magma is added to the solidification zone and undergoes crystallization there. The mean fraction f of the added magma remains as liquid

when the remaining liquid (f_A) migrates out of the solidification zone back into the magma chamber. The rest of the liquid (f_T) remains in the solidification zone, undergoes further solidification and ends up as part of the cumulate pile. The returned liquid has a different elemental concentration to the magma in the chamber because of the fractionation processes occurring in the solidification zone. Thus the liquid in the magma chamber changes composition progressively as a result of the return of interstitial liquid from the solidification zone. Note that f applies only to the solidification zone. The fraction of the initial chamber remaining liquid (commonly referred to as F in trace-element equations) is M_L/M_0 .

upward flow of intercumulus liquid, some of which could be returned to the magma chamber. Walker *et al.*¹⁴ showed that intercumulus liquid was efficiently expelled from a crystal/liquid mush in an experimental charge, giving a compaction efficiency far greater than that expected from gravitational forces. Models of compositional convection in the solidification zone¹¹ and the development of boundary-layer instabilities¹⁵ also suggest mixing of liquids from the solidification zone. Evolution of volatiles in the solidification zone might also aid transport. Compaction resulting from deformation of the matrix (for example, ref. 16) is probably too slow a process to lead to significant return of melt, but sedimentary compaction could be more efficient. Thus it seems likely that some liquid from the solidification zone will be returned to the magma chamber, although the process and efficiency may differ for floor, roof and walls. Here I show that such return of residual liquid from the magma-chamber margins could influence substantially the paths of geochemical evolution in magma chambers. If this return of liquid occurs, it may be necessary to modify many petrogenetic conclusions that have relied on fractional-crystallization models.

Qualitative effects

The solidification zone of a magma chamber is an open system that contains gradients in all physical and chemical properties. Even physical models of simple chemical systems are only at the initial stages of a quantitative understanding of the processes and exchanges that take place (for example, ref. 17). Because of these complexities, quantitative treatments will evolve considerably with time. But there are some clear qualitative consequences that will differ substantially from classical fractional crystallization, as illustrated in Fig. 2.

The first obvious effect is that some of the phases being removed are below the liquidus of the magma in the chamber. The evolution of the magma can thus reflect sub-liquidus phases, and the magma in the chamber need not evolve along cotectics on appropriate phase diagrams.

A second aspect is the relative behaviour of incompatible elements and major elements. A perfectly incompatible element will remain excluded from all crystal phases and will increase in inverse proportion to the magma-chamber volume, just as it does for fractional crystallization. But the change in major-

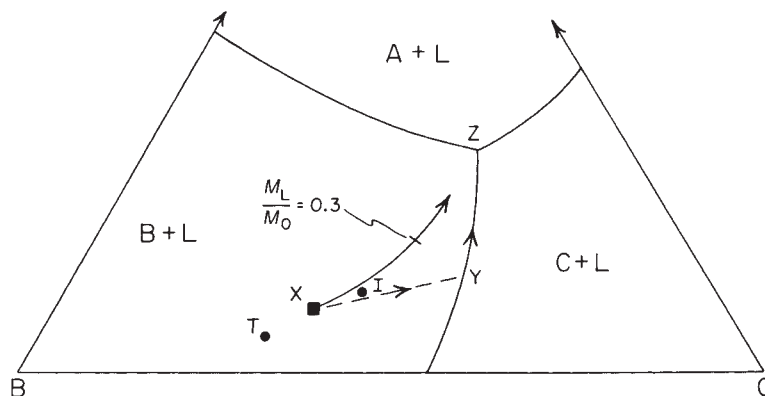
element concentration is controlled by the removal of the total solid composition, which depends on the proportions and compositions of precipitating phases. The total solid composition near the solidus is more similar to the initial liquid composition than are the solid phases on the liquidus. Thus the removal of a mean solid composition between liquidus and solidus causes the major elements usually to be changed less than they would be by removal of only liquidus phases. The net result is that *in situ* crystallization causes more change in the incompatible elements relative to the major elements than does fractional crystallization.

A third aspect is the complex relationships that are possible among the fraction of liquid remaining, the degree of evolution of the magma, and the cumulate compositions. Consider two extreme possibilities for *in situ* crystallization. One extreme is fractional crystallization, which would occur for a solidification zone with zero thickness and a flat interface, causing there to be no residual liquid. In this case there are large changes in the concentrations of most elements in both magma and cumulates as the chamber crystallizes. The cumulates are the liquidus phases of the eruptable liquids of the chamber interior at each point in its evolution. The other extreme is where there is no return of residual liquid from the solidification zone. In this case the magma never evolves—there is no change in liquid or cumulate composition as the chamber solidifies. The cumulates in the solidification zone are simply the solid equivalent of the chamber interior. Hence the cumulates are far more 'evolved' than the liquidus phases in equilibrium with the erupted liquids.

An equation for *in situ* crystallization

Despite the uncertainties in the exact processes occurring in the solidification zone, development of a simple quantitative model is of use for illustrating some of the effects of *in situ* crystallization and for applications to modelling geochemical data. The physical model evaluated here is illustrated in Fig. 1b. A 'solidification zone', in which crystallization proceeds, exists on the margins of the magma chamber. (The term 'solidification zone' is used rather than the term 'boundary layer' because the solidification zone is distinct from the thermal or mechanical boundary layers for which there are rigorous definitions.) Some liquid is expelled from the solidification zone and is returned

FIG. 2 Phase diagram for the hypothetical ternary eutectic system A-B-C. For fractional or equilibrium crystallization, bulk composition X follows the path X-Y-Z. The solidification zone (see Fig. 1) extends from solidus to liquidus and contains within it the entire liquid line of descent for equilibrium crystallization. The *in situ* crystallization path is the curved solid line originating at point X. It was calculated incrementally by mixing the various liquids in the solidification zone in proportion to their abundance with the current composition of the 'magma chamber,' then adding a new increment to the solidification zone, and so on. Abundance in the solidification zone was assumed to be a linear function of distance (for example, 0.9 units of liquid having been crystallized 10%, 0.1 units crystallized 90% and so on). This leads to a mean $f=0.5$. The points T and I mark the total and instantaneous solid compositions, respectively, that correspond to the point indicated for the *in situ* path after 70% crystallization. The three qualitative characteristics of *in situ* crystallization that are discussed in the text can be distinguished in the figure. First, the *in situ* path curves across a single primary-phase field, and the total solid composition includes phases A and C, although neither of those phases appears on the liquidus of eruptable liquids in the magma chamber. Second, the liquid that has undergone 70% *in situ* crystallization is much closer to the starting composition X than liquid that has undergone 70% equilibrium crystallization, which has reached the eutectic point, Z. An incompatible element would be enriched by a factor of 3.3 in both cases. This illustrates the greater increase in incompatibles, for comparable changes in major elements, caused by *in situ* crystallization. Third, consider some of the petrogenetic misinterpretations of volcanics that lay



along an *in situ* crystallization path. Conventional interpretations of the volcanics would suggest complex replenishment and mixing, or possibly different extents of melting as the eutectic point moves as a function of pressure. The plutonics (such as point I) would suggest cotectic or eutectic liquids that were far more evolved than those actually present in the magma chamber, and yet would have phase proportions that do not correspond exactly to the relevant phase diagram. An erroneous pressure of crystallization, or complex boundary-layer processes, might be invoked to explain the discrepancies.

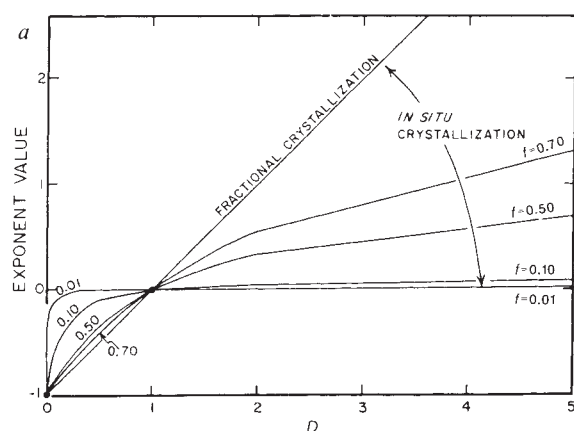
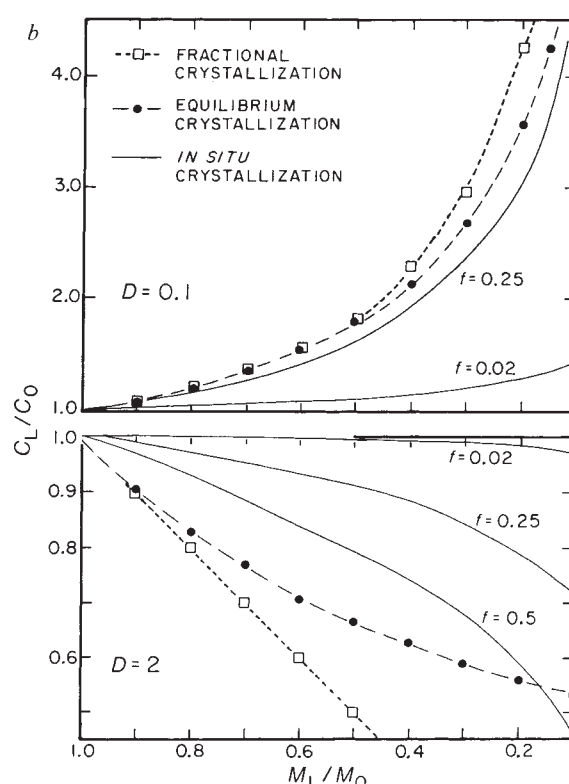


FIG. 3 a, Comparison of the exponent of the *in situ* crystallization equation (6) with the exponent of the Rayleigh fractionation equation as a function of the mineral/melt partition coefficient, D , assuming a process of equilibrium crystallization in the solidification zone. The different curves are for different values of f_A in equation (6), with no trapped liquid, so that $f_A = f$. Trapped liquid would cause the *in situ* exponent to be closer to zero for all values of D . Note that *in situ* crystallization causes the effective D s for all elements to approach a value of 1 (exponent value of 0) for any D not equal to zero. b, Comparison, for two values of D ($D=0.1$ and $D=2$), of *in situ* crystallization with equilibrium crystallization and fractional crystallization. The evolution of liquid compositions in the magma chamber is shown as a function of the fraction of the magma chamber solidified, M_L/M_0 . Note the change in scales in the two parts of the figure. For $D=0$, all three processes would follow a single curve (not shown) which is inversely proportional to M_L/M_0 . The solid curves are for *in situ* crystallization for different values of f . Elements with $D=0.1$ increase much less during *in situ* crystallization if $f < 0.3$. Major elements with $D \approx 2$ can stay much more constant even when



f is as large as 0.5. Note that *in situ* crystallization with $f=1.0$ corresponds exactly with the fractional crystallization curve (open squares) in both parts of the diagram.

to the well mixed interior of the magma chamber. The composition of the returned liquid is distinct from the magma in the interior of the magma chamber because of the chemical fractionation that occurs in the solidification zone.

The magma chamber has an initial mass M_0 . Magma (dM_I) is added to the solidification zone and fractionated liquid (dM_A) returns from the solidification zone. The mean amount of solidification of the added liquid before it is expelled is $1-f$, where f is the fraction of the original, added magma that remains liquid when expulsion occurs. Not all the liquid will escape, so $f = f_A + f_T$, where f_A is the portion of f that returns to the magma chamber, and f_T the portion that remains in the solidification zone. Note that f_T is not exactly trapped liquid; trapped liquid is a concept that applies best to crystal settling. The proportion of liquid that remains after expulsion is $f_T/(1-f_A)$, which is always a larger number than f_T .

The mass of interstitial liquid returned to the magma chamber is related to the mass of magma added to the solidification zone by

$$dM_A = f_A dM_I \quad (1)$$

Then, from mass balance, the change in the mass of liquid in the magma chamber (dM_L) is

$$dM_L = -dM_I + dM_A = dM_I(f_A - 1) \quad (2)$$

The concentrations of an element in the magma chamber and returning interstitial liquid are C_L and C_I respectively. The total mass of any element in the chamber is $M_L C_L$. The change in that mass is $d(M_L C_L)$, also equal to $-C_L dM_I + C_I dM_A$. Thus,

$$C_L dM_L + M_L dC_L = -C_L dM_I + C_I dM_A \quad (3)$$

To evaluate this equation one must have a relationship between C_L and C_I . At any stage in the solidification of the magma chamber for any element, we can define the ratio E such that

$$E = C_I / C_L \quad (4)$$

E is analogous to the bulk distribution coefficient (D) conventionally used for modelling crystallization and melting. D describes the instantaneous fractionation between liquid and crystals, whereas E describes the instantaneous fractionation between the two liquids. Whereas fractional crystallization occurs by separation of crystals with fractionation controlled by D , *in situ* crystallization occurs by addition of liquids with fractionation controlled by E .

Substitution from (1), (2) and (4) into (3) leads to

$$\frac{dC_L}{C_L} = \frac{dM_L}{M_L} \left[\frac{f_A(E-1)}{f_A-1} \right] \quad (5)$$

Equation (5) would be readily integrable if f_A and E were constant throughout solidification, but in practice this is not likely, and so for quantitative modelling it is desirable to proceed numerically; these results follow in later sections of this paper. But this situation is analogous to having a constant D during fractional crystallization and melting, and it is clear that the integrated equations made possible by constant D can nonetheless be very useful, particularly for understanding the general effects of the process and for gaining intuition. The same is true for equation (5). So for the case where E and f_A are constants, one can integrate with the initial condition that $C_L = C_0$ when $M_L = M_0$, to give

$$\frac{C_L}{C_0} = \left(\frac{M_L}{M_0} \right)^{(f_A(E-1)/(f_A-1))} \quad (6)$$

M_L/M_0 is equivalent to the fraction of liquid remaining, normally denoted as F . Note the similarity in the form of this *in situ* crystallization equation to the Rayleigh fractionation equation ($C_L/C_0 = (M_L/M_0)^{D-1}$). The exponent, in equation (6), however, is a very different quantity.

Note that developing the problem in this way makes no assumptions about the nature of the process in the solidification zone, nor does it assume the existence of a homogeneous liquid

there. The crucial factor is the net effect of all the complex processes, which ultimately results in a net difference in composition between liquid added to the solidification zone and liquid returned from it, reflected by the fractionation factor E .

The exponent in (6) can also be written as $(f - f_T) \times (E - 1) / (f - f_T - 1)$. A finite value of f_T causes the exponent to be closer to zero than it would be without the f_T term. As f_T approaches f , the exponent approaches zero. Then, no liquid is returned to the magma chamber, and no differentiation occurs as the chamber solidifies.

Equation (6) exhibits the qualitative effects expected from the process discussed above. If there is no trapped liquid ($f_A = f$), then for an incompatible element, $E = 1/f$, and the exponent in (6) reduces to -1 , as in fractional crystallization. For compatible elements, however, E does not become large as f becomes small, and the exponent in (6) goes to zero as f goes to zero. No change of the compatible-element concentration occurs during solidification of the magma chamber. Thus incompatible

elements increase in the liquid portion of the magma chamber in inverse proportion to the magma-chamber volume, but major-element compositions remain much more constant.

Comparison to other fractionation processes

It is instructive to be able to compare the consequences of the *in situ* crystallization equation with other crystallization processes for a wide variety of elements. To do this, it is useful to relate the fractionation factor E to the distribution coefficient D . This can be done if one makes an assumption about the process that occurs in the solidification zone. I assume here that equilibrium crystallization occurs in the solidification zone but then there is still a range of liquid compositions, and the question is how the composition of the mixture of liquids is related to the mean fraction of liquid, f . Plank and Langmuir¹⁸ have shown recently in another context that when the D s are constant there is little difference in composition between such mixed liquids and a single liquid derived by equilibrium crystallization to the mean f . Hence the value of E may be approximated by $1/[D(1 - f) + f]$, where f is the mean of the mixture of liquids returned from the solidification zone. Then, for the case of no trapped liquid, the exponent in (6) becomes $f(D - 1)/[D(1 - f) + f]$. As f approaches 1, the exponent becomes $D - 1$, and equation (6) reduces to perfect fractional crystallization. The same result occurs as f approaches 1 if $E = f^{D-1}$. Physically this reflects the case in which, as each crystal grows, all the liquid around it is expelled immediately.

With equation (6) in this form, one can effectively compare it with the Rayleigh fractionation equation by seeing how the values of the exponents vary as a function of D (Fig. 3a). The exponents are the same only when D is 0 or 1. Between 0 and 1 the values of the *in situ* exponent are larger, whereas for $D > 1$ the values of the *in situ* exponent are smaller. In general, *in situ* crystallization causes small changes in concentration of elements with $D > 1$ (such as most major elements). Note also that for small D the slopes of the curves in Fig. 3a are steeper for *in situ* crystallization than for fractional crystallization; thus two incompatible elements with only minor differences in D are separated from one another more efficiently. Figure 3b shows the more conventional diagram, C_L/C_0 against M_L/M_0 , which allows a comparison with equilibrium crystallization as well as with fractional crystallization.

Periodically replenished magma chambers. O'Hara² and O'Hara and Mathews⁴ calculated the effects of periodically replenished magma chambers (PRMCs) on magmatic evolution, assuming constant distribution coefficients between crystals and liquid. Two of their conclusions were that the chemistry of erupted liquids would "resemble closely the expected behaviour in primary magmas generated by partial melting of a uniform source region"; and that "significant changes can occur in the ratio of highly incompatible and moderately incompatible elements ($D \approx 0.01-0.2$)". These statements resemble the effects of *in situ* crystallization, and thus the effects of PRMCs need to be evaluated carefully to see if it is possible to distinguish them from *in situ* crystallization. The two statements quoted above were made for the case where no assimilation was occurring, and that case will be considered here.

An important aspect of PRMCs that has perhaps not been recognized sufficiently is that when eruption occurs after fractional crystallization in the magma chamber, steady-state compositions lie on the closed-system, equilibrium-liquid line of descent of the parental magma supplying the magma chamber, if x (the fraction solidified) and y (the fraction erupted) are small^{3,4,19}. As x and y increase, the steady-state composition varies smoothly between the closed-system liquid line of descent for equilibrium and fractional crystallization³. This is shown in Fig. 4a. Consider closed-system crystallization of the bulk composition, P , which contains a minute amount of anorthite (0.1%). For equilibrium crystallization, the liquid moves from P to Q to B' . At B' , 90% crystallization has occurred,

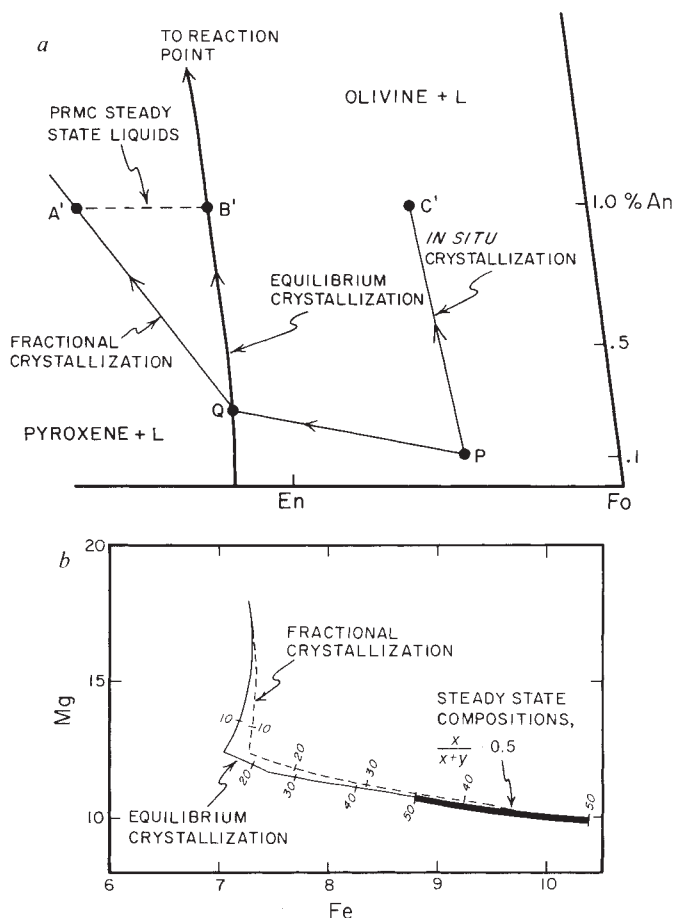


FIG. 4 a, Schematic phase diagram (forsterite-anorthite-silica) modified after O'Hara and Mathews⁴. Point P is the starting composition. Fractional crystallization of P goes to Q and then A'. Equilibrium crystallization of P goes to Q and then to point B'. All steady-state compositions with 1% anorthite from a periodically replenished magma chamber lie between A' and B'. *In situ* crystallization can follow a path such as P-C', along which the forsterite/enstatite normative ratio changes much less than is the case for fractional crystallization in either closed or periodically replenished systems. b, Plot of Mg against Fe in cation mol% for a periodically replenished magma chamber using a picritic parental composition. The small numbers on the curves indicate per cent solidified. x and y refer to the fraction solidified and erupted in each cycle of a replenished magma chamber. The diagram was calculated using the liquid-line-of-descent program of ref. 28, and illustrates that steady-state products of a periodically replenished magma chamber lie between the equilibrium and fractional liquid lines of descent in the closed system even when solid solutions are present and when the partition coefficients change as a function of temperature and liquid composition.

so anorthite has increased to 1%. For fractional crystallization, the liquid goes to Q and then to point A', which also contains 1% anorthite. All steady-state compositions with 1% anorthite lie between B' and A'. That is, they lie between the two end-members of closed-system crystallization.

This relationship of steady-state products of PRMCs to closed-system liquid lines of descent is important because two simple, closed-system calculations for equilibrium and fractional crystallization constrain the potential compositions of eruptive products. Steady-state compositions after crystallization lie on or between the closed-system liquid lines of descent. Steady-state compositions after mixing are constrained to mixing lines between the parental magmas and the closed-system liquid lines of descent. This conclusion is not restricted to the cases of constant D s and simple phase diagrams, but is also true in general for both major and trace elements, even when D s change as a function of composition and temperature and multiple solid solutions are present. This becomes apparent when liquid-line-of-descent calculations are applied to PRMCs (Fig. 4b).

Figures 4a and 5 compare this behaviour of PRMCs with *in situ* crystallization. In Fig. 4a, if f is small, the liquid in the solidification zone undergoing equilibrium crystallization continues to evolve along the reaction curve past B', and towards the olivine–enstatite–anorthite invariant point, which is well off the top of the figure. These liquids mix progressively with the parental composition, causing the magma in the chamber to evolve more directly towards the reaction point. When the liquid reaches point C', the anorthite content of the liquid has increased to 1%, as in the PRMC examples, but in contrast to the PRMCs the normative olivine/pyroxene ratio, and hence major-element composition, has changed very little. Figure 5 shows that PRMCs also do not replicate the effects of partial melting of the mantle.

Figures 4 and 5 allow a re-evaluation of O'Hara's two conclusions quoted above. When the fractions erupted and crystallized are very small, the steady-state products may be technically the same as those derived by equilibrium crystallization (or melting), but the composition that was melting would be that of the magma itself, which is not at all the same thing as partial melting of the source region. The separation of major and trace elements which is possible in the closed system by the *in situ* process is not possible in replenished magma chambers undergoing fractional crystallization.

Although the geochemical importance of PRMCs has been overstated^{2,4}, the geological arguments in favour of replenish-

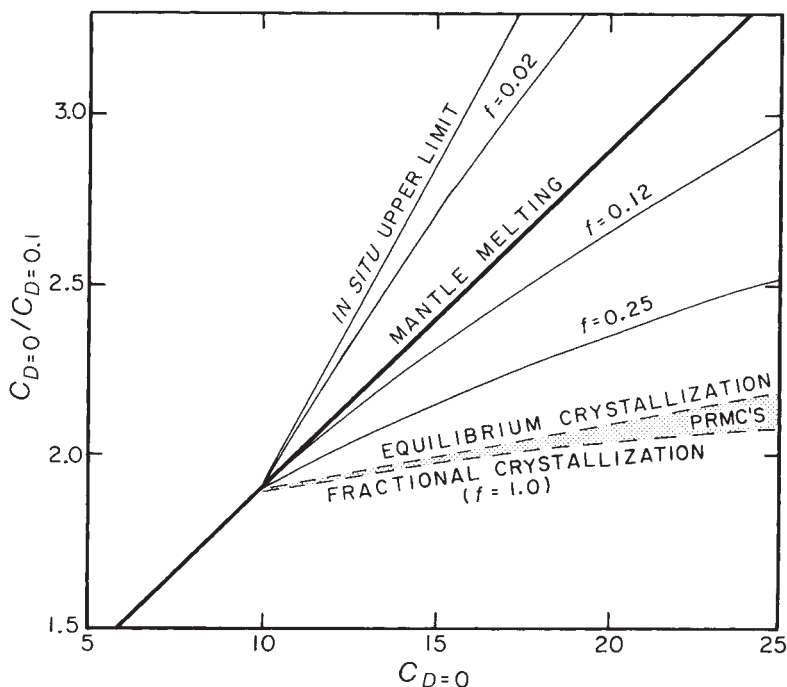
ment of magma chambers^{2,20} are obviously independent of the crystallization process. As the *in situ* crystallization equation (6) has the same mathematical form as Rayleigh fractionation, the steady-state equations for replenishment are exactly analogous except that the exponent is that in equation (6).

Applications to real rocks

The practical significance of fractionation by return of residual liquid depends very much on the values of f_A and E that actually occur. The most extreme fractionation between major and incompatible trace elements occurs for small values of f . But such values may be unlikely because the small amounts of liquid from the outer edges of the solidification zone are likely to mix with less crystallized portions of the zone during transport to the chamber interior. The important factor then becomes the distribution and means of transport of the various compositions in the solidification zone. If the amount of liquid varies linearly with distance in the solidification zone, then $f=0.5$. Such a value of f can separate to some extent the major elements from incompatible trace elements (see Fig. 2) and can transmit the effects of phases from below the liquidus, but would not fractionate one incompatible trace element from another. For results similar to melting of the source region, values of f would have to be below 0.2, which would require large volumes of mostly crystalline mush from which the liquids were efficiently removed. A realistic evaluation of these values is difficult, because so little is known about the complex processes at the magma-chamber margin. One way to address the questions of the importance of the return of liquid and the probable values of f is to apply the *in situ* crystallization model to layered intrusions and suites of volcanic rocks, to see if it helps to explain data that otherwise is somewhat enigmatic.

The Kiglapait layered intrusion. Morse²¹⁻²⁶ has studied the Kiglapait intrusion for the past twenty years and has found exposures of the intrusion from a basal contact up to very near its roof. This has allowed him to estimate the bulk composition of the intrusion from chemical analyses of the cumulates. He suggests that the intrusion was formed by closed-system fractional crystallization from magma of the calculated bulk composition, and that the trapped liquid component was small. For our purposes it will be assumed that, as Morse has suggested, the intrusion was formed by crystallization in a closed system from a known bulk composition, at least up to ~85% crystallization, even though this supposition has been questioned by

FIG. 5 Plot of the ratio of a highly incompatible element with $D=0$ to a moderately incompatible element with $D=0.1$ against the abundance of the highly incompatible element (see, for example, ref. 42), to compare periodically replenished magma chambers, *in situ* crystallization and partial melting of the mantle. The heavy diagonal line represents melting of a source with unit concentrations. All calculations of crystallization processes were done with a starting composition derived by 10% melting. The thin solid lines show the effects of *in situ* crystallization of such a melt. Note that, if $f < 0.1$, *in situ* crystallization can cause still greater changes in a ratio of two incompatible elements than can different extents of melting (of the mantle, for example). Dashed lines show the effects of equilibrium and fractional crystallization. Steady-state products of periodically replenished magma chambers (PRMCs) after crystallization are confined between the two dashed lines, and hence do not replicate the effects of partial melting of the mantle.



others²⁷. Accepting the assumption of a closed system and the calculated liquid compositions²⁶, the variations in mineral chemistry, mineral proportions and liquid composition can be compared with the models of fractional and *in situ* crystallization. The following discussion is based on liquid-line-of-descent calculations carried out using the program of Weaver and Langmuir²⁸, which calculates liquid lines of descent involving olivine, plagioclase and clinopyroxene for dry systems at 1 atm pressure. These are the only cumulus minerals in the Kiglapait up to about 85% solidification.

The lowermost cumulates of the Kiglapait are olivine/plagioclase accumulates^{23,24}. Calculation of the equilibrium phases for the Kiglapait bulk composition immediately reveals inconsistencies if a fractional-crystallization model is used. First, plagioclase is calculated to be alone on the liquidus over a substantial temperature interval. The pressure difference cannot account for this discrepancy, because the pressure effect on the cotectic is small²⁴. Second, the mineral compositions are incorrect—the observed olivine is too iron-rich (~Fo68 as opposed to Fo83) and the plagioclase too sodic (~An65 as opposed to An80). Calculation of the evolution of the liquid composition during fractional crystallization also shows marked discrepancies with respect to the Kiglapait liquid, as shown in Fig. 6. Fractional crystallization causes more rapid changes as a function of M_L/M_0 .

Paths of liquid evolution for *in situ* crystallization are shown in Fig. 6. The liquid evolution for *in situ* crystallization matches

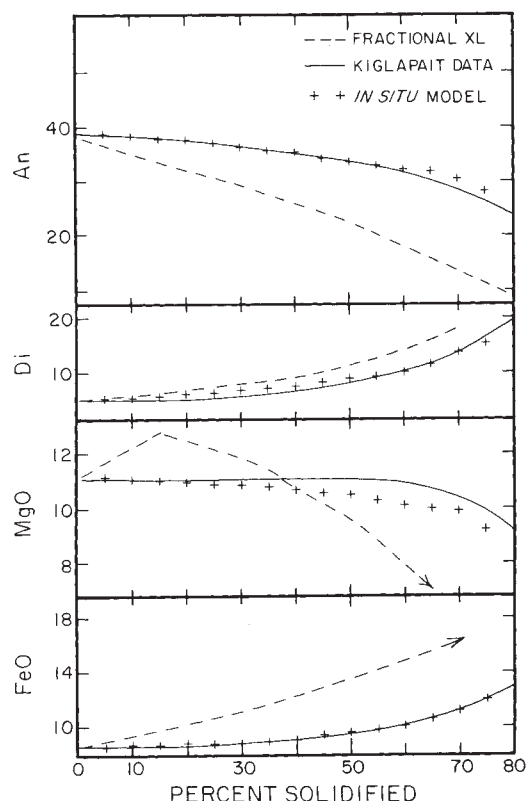


FIG. 6 Comparison of liquid lines of descent for fractional (dashed line) and *in situ* (crosses) crystallization with the liquid evolution of the Kiglapait intrusion presented by Morse²⁶ (solid line). The *in situ* crystallization model was calculated incrementally using the program of ref. 28, for a value of $f=0.25$ with no trapped liquid. Note that these are numerical calculations for which E changes as solidification proceeds, and as different phases appear on the liquidus; thus the paths are not calculated using equation (6). An and Di are cation-normative anorthite and diopside, respectively. MgO and FeO are expressed in cation mol%.

the observed trends much more closely than does fractional crystallization. After about 70% solidification, the *in situ* compositions for $f=0.25$ begin to deviate from the measured magma-chamber composition. A gradually increasing value of f is required to match the data, that is, the magma-chamber composition more closely approaches that expected from fractional crystallization as the fraction of magma remaining in the chamber becomes small.

The *in situ* process also accounts for the anomalies in phase appearances, compositions and proportions mentioned above. As the interstitial liquid is derived from a solidification zone that is 75% crystallized, the phase proportions and compositions should reflect, not the liquidus-phase proportions, but those at equilibrium with the liquid after 75% equilibrium crystallization. As plagioclase is alone on the liquidus over an interval of 40 K, the phase proportions after 75% crystallization have significantly more plagioclase than the cotectic phase proportions. Indeed, the calculated phase proportions are similar to those observed (78% as opposed to 79% plagioclase). The equilibrium minerals also are less calcic and less magnesian after 75% equilibrium crystallization than the liquidus minerals, and the calculated mineral compositions are similar to those observed (An63 versus An65, Fo65 versus Fo68). Thus, despite the oversimplifications of the modelling, that is, neglecting the variations in composition and the complex processes that must be occurring in the solidification zone, the *in situ* process nonetheless accounts remarkably well for the liquid evolution, phase chemistry and phase proportions of the Kiglapait intrusion, whereas none of these is accounted for by fractional crystallization for the first 80% solidification of the intrusion.

The modelling works well because *in situ* crystallization invariably causes the removal of a mean solid composition comprising several phases—and hence a smooth liquid line of descent that does not depend only on liquidus minerals—and because it causes major elements to change much less as a function of M_L/M_0 than does fractional crystallization.

Volcanic rocks from divergent and convergent plate margins.

Two of the qualitative geochemical signatures of *in situ* crystallization are, first, evidence for the removal of low-temperature phases below the liquidus of the erupted lavas, and second, greater increases of incompatible elements with fractionation than seems possible by fractional crystallization. These characteristics are common to many suites of volcanic rocks. Mid-ocean-ridge basalts exhibit the 'pyroxene paradox'²⁹, according to which mass balance requires pyroxene removal, yet none is on the liquidus. Walker³⁰ has noted a similar problem for mare basalts on the moon with missing plagioclase. Excesses of trace-element enrichment with increasing fractionation are also almost ubiquitous for mid-ocean-ridge basalts (see, for example, refs 31–33). For continental lavas, Carmichael *et al.*³⁴ pointed out that magnetite fractionation was often not an adequate explanation of calc-alkaline differentiation because of the absence of magnetite from the liquidus of erupted lavas. But both magnetite and hornblende could occur in the solidification zone, so that their absence from the liquidus does not necessarily imply that they play no role in causing the calc-alkaline trend.

Conclusions

Even if *in situ* crystallization, as modelled crudely here, is not the only, or even the dominant, process (see, for example, ref. 35) causing differentiation in magma chambers, any re-injection of liquid from the solidification zone may lead to paths of chemical evolution that are very different from fractional crystallization in closed or periodically replenished magma chambers. This may necessitate a reconsideration of some of the petrogenetic conclusions that are based on constraints from models of fractional crystallization. For example, apparent removal of sub-liquidus phases and the presence of evolved phenocryst assemblages have been used as evidence for mixing; but they may also be a consequence of normal evolution by *in*

situ crystallization in a closed system. Cumulate compositions are sometimes used to estimate coexisting liquids; but the cumulate composition may be far removed from erupted compositions. Substantial variations in incompatible trace elements for constant major elements have been used as evidence of variations in the extent of partial melting; but this is a general characteristic of the *in situ* process. As the chemical consequences of *in situ* crystallization become better understood, it will be necessary to develop tests to determine how many of these features of igneous petrogenesis it actually does account for. It is possible that the thermal conditions and density relationships for different tectonic settings could cause fundamentally

different evolutionary paths as a result of the nature of return of residual liquid from the solidification zone.

If further work confirms the importance of the process, subsequent efforts will need to take account of the vertical zonation of many magma chambers (see, for example, refs 12, 36–40), the variations in liquid compositions in the solidification zone, interaction with pre-existing crystals as the liquid moves through the solidification zone, and kinetic effects. Although this paper has focused on magma chambers, it is possible that a similar process may lead to complex changes in liquid composition as a magma moves through a conduit system with a partially molten solidification zone. □

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Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast

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Conjugative plasmids of *Escherichia coli* can mobilize DNA transmission from this bacterium to the yeast *Saccharomyces cerevisiae*. The process shares some of the features of conjugation between bacteria and could be evolutionarily significant in promoting trans-kingdom genetic exchange.

TRANSMISSION of genetic information between distant taxonomic groups could create organisms with unique evolutionary potential. One well-studied example of such gene transmission occurs between the bacterium *Agrobacterium tumefaciens* and certain plant species (for review, see ref. 1). It seems that DNA transfer from *A. tumefaciens* to plants is mechanistically similar to bacterial conjugation^{2,3}. Most strikingly, conjugative plasmids can substitute for tumour-inducing plasmid functions necessary for transmission of DNA to plants, provided that certain tumorigenic-encoded virulence functions that contribute to DNA transfer are also present⁴. Here we investigate whether bacterial conjugation could provide a gen-

eral mechanism for inter-kingdom genetic exchange using derivatives of two classes of bacterial conjugative plasmids: broad-host-range plasmids, such as R751, which promote conjugation between a large number of bacterial species, including between Gram-negative and Gram-positive species^{5–7}, and the F plasmid (fertility factor), which confers a limited-host-range phenotype, its conjugation being limited to *E. coli* and related Enterobacteriaceae⁸. Here we show that both broad-host-range and limited-host-range conjugation plasmids can transmit DNA from bacteria to yeast.

Transfer of plasmids between bacteria by conjugation requires cell contact and DNA metabolism^{9–12}. Transmission to the new host also requires that the plasmid should replicate, either autonomously or by association with a host replicon. The donor cell in a conjugal relationship contains a self-transmissible plasmid which encodes the capacity to carry out the transfer functions. The plasmid *tra* locus encodes cell-surface components as well as mobilization proteins (*mob* gene products) which promote DNA transfer by interaction with a *cis*-acting sequence termed the origin of transfer (*oriT*). We use representatives of three different plasmid groups: R751, ColE1 and the fertility factor F. For simplicity, suffixes are used to denote the origin

of conjugation functions: for example, *mob*-R and *oriT*-R refer to the *mob* and *oriT* functions of the R751 group respectively (Table 1).

DNA transmission from bacteria to yeast

To determine whether plasmids can be transmitted from bacteria (*E. coli*) to yeast (*S. cerevisiae*) by conjugation, we have used two bacterial strains, one containing both a self-transmissible plasmid and a plasmid that can replicate and be selected in yeast, and the other containing only the yeast plasmid. The plasmid pDPT51, a derivative of the broad-host-range plasmid R751, contains *mob*-R and *oriT*-R for self-transmission and also the *mob*-C region from ColE1 (Table 1). Thus, pDPT51 can mobilize *in trans* other plasmids, such as pBR322, that contain the *oriT* region from ColE1 (*oriT*-C, or the *bom* site⁹). We used the yeast plasmid YEp13 (Table 1), which is a yeast-bacteria shuttle vector derived from pBR322. YEp13 carries the yeast *LEU2* gene and a segment of the yeast 2 μ plasmid, allowing selection and replication of the plasmid in yeast.

We tested *E. coli* cells containing both plasmids for the ability to transmit plasmid DNA to yeast cells and to other *E. coli* strains using a standard protocol for bacterial conjugation (see Table 2 legend). When donor bacteria are mixed with *leu2*⁻ yeast cells, *Leu*⁺ yeast are detected at a frequency of 3×10^{-7} *Leu*⁺ cells per recipient (Table 2a). We confirmed that the *Leu*⁺ yeast cells contained YEp13 by restriction-endonuclease analysis of plasmid DNA recovered from these cells. Although we

presume that pDPT51 was also transferred to yeast, we did not detect its transmission because it lacks a replication sequence for propagation in yeast.

The frequency of transmission of YEp13 ranges from 3×10^{-8} to 5×10^{-5} *Leu*⁺ cells per recipient and can be as high as 7×10^{-2} *Leu*⁺ cells per donor, depending partly on the ratio of donor to recipient cells in the mating assay (Tables 2 and 3). In bacterial conjugation experiments, transconjugant bacteria arose at a frequency of $\sim 3 \times 10^{-1}$ transconjugants per recipient (Table 2). Initially we made no attempt to optimize the ratio of bacteria to yeast in the mating assay, but later found that an excess of bacterial cells reduced the frequency of DNA transmission, which has been found for conjugation between bacteria⁹. The donor:recipient ratios used in each experiment are given in Tables 2 and 3.

To see whether the appearance of *Leu*⁺ yeast cells was dependent on the conjugative plasmid, *E. coli* carrying only YEp13 was mixed with yeast. No *Leu*⁺ yeast was observed ($< 1 \times 10^{-9}$ *Leu*⁺ cells per recipient; Table 2a), indicating that DNA transmission was dependent on a conjugation plasmid. We therefore

TABLE 1 Plasmid functions

Plasmid	Conjugation functions	Selectable markers* in bacteria	in yeast	Refs
R751	<i>tra</i> -R, <i>mob</i> -R, <i>oriT</i> -R	Tp ^r	none	(19)
pDPT51	<i>tra</i> -R, <i>mob</i> -R, <i>oriT</i> -R, <i>mob</i> -C	Tp ^r , Am ^r	none	(20)
pDPT51:LEU2	<i>tra</i> -R, <i>mob</i> -R, <i>oriT</i> -R, <i>mob</i> -C	Tp ^r , Am ^r	<i>LEU2</i>	This work
YEp13	<i>oriT</i> -C	Tc ^r , Am ^r	<i>LEU2</i>	(21)
YEp24	<i>oriT</i> -C	Tc ^r , Am ^r	<i>URA3</i>	(22)
pAL2	none	Cm ^r	<i>LEU2</i>	This work
pACYC184	none	Cm ^r , Tc ^r	none	(15)
pFLEU2	<i>tra</i> -F, <i>mob</i> -F, <i>oriT</i> -F	Am ^r	<i>LEU2</i>	This work
pRS2405	<i>tra</i> -F, <i>mob</i> -F, <i>oriT</i> -F	Am ^r	none	(23)

Plasmids R751, pDPT51, YEp13, YEp24, pACYC184, and pRS2405 have been described (refs 15, 19–23). Plasmids R751 and pDPT51 are broad-host-range conjugative plasmids of the P incompatibility group. Plasmid pRS2405 is a limited-host-range plasmid of the F incompatibility group. pAL2 was constructed by cloning an *Xho*I–*Hind*III fragment that contains *LEU2* and 2 μ sequences from YEp13 into the *Sal*I and *Hind*III sites of pACYC184, resulting in the deletion of the *tet*^r gene of pACYC184. pFLEU2 and pDPT51:LEU2 were constructed in multiple steps. First, the *Xho*I–*Hind*III 2 μ , *LEU2* fragment of YEp13 was cloned into the *Sal*I–*Hind*III sites of pUC12 (ref. 24). The 2 μ , *LEU2* fragment was then cloned as an *Eco*RI (from the pUC12 polylinker)–*Hind*III fragment into the *Eco*RI and *Hind*III sites of pBR322 (ref. 25). In the resulting plasmid, the 2 μ , *LEU2* segment is flanked by *Bam*HI sites, one from the pUC polylinker and one in the *tet*^r gene of pBR322. To create pFLEU2, the *Bam*HI fragment was inserted into the *Bam*HI site of pRSF2405. To create pDPT51:LEU2, the *Bam*HI fragment was first cloned into the *Bam*HI site of pNK1207 (ref. 26), a derivative of pBR333. The resulting plasmid was integrated into pDPT51 by recombination *in vivo* to yield pDPT51:LEU2. All plasmids other than R751, pDPT51, pACYC184 and pRS2405 contain yeast 2 μ -plasmid sequences which permit autonomous replication in yeast.

* The plasmids contain genes which confer resistance to antibiotics in bacteria. Tp^r indicates that bacteria harboring the plasmid are resistant to trimethoprim; Am^r, resistance to ampicillin; Tc^r, resistance to tetracycline; and Cm^r, resistance to chloramphenicol. In addition, some plasmids contain the yeast *LEU2* or *URA3* genes. These plasmids can be selected in yeast because they complement nutritional auxotrophies conferred by mutations in the chromosomal *LEU2* or *URA3* genes.

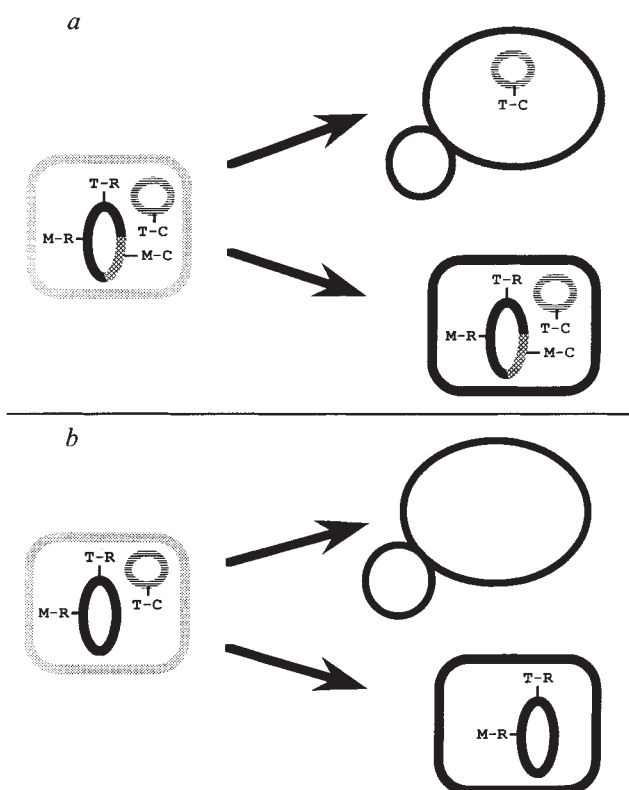


FIG. 1 Representation of the requirement for appropriate mobilization functions for plasmid transmission to bacteria and to yeast, as interpreted from results presented in Table 3, a1 and a2. The large rounded squares represent bacteria; donor bacteria are depicted with stippled borders and recipient bacteria with solid borders. The large budded circle represents yeast. Circles and ellipses within the cells represent plasmids. The ellipse with the solid border is plasmid R751. Plasmid pDPT51 is a derivative of R751 that carries the *mob*-C region (depicted by wide cross-hatching) from ColE1. The circle with the horizontally-striped border represents the bacteria–yeast shuttle vector YEp13. The *mob* and *oriT* functions present on the plasmids are represented by abbreviations: M-R, *mob*-R; T-R, *oriT*-R; M-C, *mob*-C; T-C, *oriT*-C. a, The *mob*-C function of pDPT51 acts at the *oriT*-C of YEp13 and promotes its transmission to both bacteria and yeast. The *mob*-R function of pDPT51 acts at the *oriT*-R present on the same plasmid and promotes its transmission to bacteria. In principle, pDPT51 may also be transferred to yeast, but the plasmid lacks functions that permit replication and selection in yeast, so transfer cannot be detected. b, Plasmid R751 is transmitted to bacteria by means of its *mob*-R and *oriT*-R functions. However, YEp13 is not transmitted to either bacteria or yeast because no *mob*-C function is present in the donor bacterium. Again, R751 may also be transferred to yeast but this is undetectable.

TABLE 2 Physical requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Treatment	Transconjugant cells per ml	Donor: recipient ratio	Transconjugant cells per recipient
a					
YEp13	Y	none	0	30:1	$<1 \times 10^{-9}$
pDPT51, YEp13	Y	none	42	6:1	3×10^{-7}
b					
None	Y	YEp13 DNA	0	40:1	$<5 \times 10^{-9}$
pDPT51	Y	YEp13 DNA	0	30:1	$<1 \times 10^{-9}$
pDPT51, YEp13	Y	none	73	6:1	1×10^{-7}
No bacteria	Y	YEp13 DNA	0	—	$<2 \times 10^{-9}$
c					
pDPT51, YEp13	Y	CHCl ₃	0	—	$<5 \times 10^{-10}$
pDPT51, YEp13	Y	CHCl ₃ buffer	15	3:1	2×10^{-7}
pDPT51, YEp13	Y	none	11	1:2	3×10^{-8}
d					
pDPT51, YEp13	Y	pellet	3×10^2	1:300	3×10^{-6}
pDPT51, YEp13	B	pellet	2×10^6	1:30	2×10^{-1}
pDPT51, YEp13	Y	supernatant	0	—	$<2 \times 10^{-8}$
pDPT51, YEp13	B	supernatant	0	—	$<2 \times 10^{-7}$
e					
pDPT51, YEp13	Y	separated by filter	0	—	$<5 \times 10^{-9}$
pDPT51, YEp13	B	separated by filter	0	—	$<5 \times 10^{-10}$
pDPT51, YEp13	Y	combined on filter	1×10^4	1:30	5×10^{-5}
pDPT51, YEp13	B	combined on filter	2×10^8	1:2	3×10^{-1}

Donor and recipient cells were prepared for conjugation by a procedure adapted from Sistrom *et al.*²⁷. Exponential cultures of donor bacteria growing in medium selective for plasmid maintenance were collected by centrifugation, washed with TNB (0.05 M Tris, pH 7.6, 0.05% NaCl), and resuspended in TNB (10^5 – 10^8 cells per ml). Similarly, exponential cultures of recipient cells growing in rich medium (LBH (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 1 mM NaOH)²⁸ for bacteria, or YEPD (2% peptone, 1% yeast extract, 2% glucose)²⁹ for yeast) were collected and resuspended in TNB (10^8 cells per ml). Donor and recipient were mixed in the ratios indicated and plated as described below. Reported results are from representative experiments. Experiments *a*, *b* and *c* used bacterial strain RR1 (*proA2 leuB6 hsdS20 thi ara14 galK2 xyl5 mtl1 lacY1 supE44 rpsL20*; ref. 25) as the donor, and yeast strain DC5 (*MATa leu2-3 leu2-112 his3 gal2 can1*) as the recipient. Experiments *d* and *e* used bacterial strain SB21 (*hsdS leuB6 thr*, a derivative of C600; ref. 30) as the donor, SY1229 (*MATa leu2-3 leu2-112 his3 gal2 can1 ura3*) as the yeast recipient, and RR1 as the bacterial recipient. Two other yeast strains have also served as recipients, one of which is only distantly related to DC5 and SY1229. Experiment *a* established that the formation of Leu⁺ yeast was dependent on a bacterial conjugation plasmid. The mixture of donor and recipient was plated directly on supplemented yeast minimal medium (SD (10.67% yeast nitrogen base, 2% glucose) + his (20 µg ml⁻¹ L-histidine); ref. 29). The number of transconjugants per ml was calculated on the basis of the volume of the mixture applied to the solid medium. No reversion to Leu⁺ was detected. Incubation of the conjugation mixture on non-selective medium before plating gave comparable frequencies of Leu⁺ transconjugants. Experiment *b* demonstrated that extracellular YEp13 DNA did not yield Leu⁺ yeast. The donor-recipient mixture was preincubated on solid non-selective medium for 5–12 hours, resuspended in TNB, and plated on SD + his. The number of transconjugants per ml was calculated on the basis of their concentration in this final suspension of TNB. The concentration of YEp13 DNA in the mixture was 6 µg ml⁻¹. Experiment *c* showed that the formation of Leu⁺ yeast was dependent on viable donor cells. The donor-recipient mixture was treated as in experiment *b*. Experiment *d* demonstrated that the formation of Leu⁺ yeast was not caused by an extracellular diffusible factor. Pelleted donor cells or the supernatant of the donor culture was mixed with the yeast recipient and plated directly on SD + his + ura (20 µg ml⁻¹ uracil). All bacterial matings (also in Table 3) were first plated on LBH to allow expression of the antibiotic-resistant phenotype. After 5 h the cells were resuspended and plated on OMBG (minimal agar plates; ref. 31) plus proline (20 µg ml⁻¹), leucine (20 µg ml⁻¹) and trimethoprim (Tp) (200 µg ml⁻¹) and tetracycline (Tc) (20 µg ml⁻¹). The frequency of transconjugant bacteria reflects the number of bacteria that received both plasmids. The same frequency was observed when transmission of only YEp13 was monitored. Experiment *e* confirmed that the formation of Leu⁺ yeast required cell-to-cell contact. The mixture was preincubated on solid non-selective medium for 5–12 h and then plated on SD + his + ura. The bacterial matings were performed as in experiment *d*.

* Y indicates that the recipient organism was yeast; B indicates that the recipient organism was bacteria.

refer to the Leu⁺ yeast obtained by co-culture with bacteria as 'transconjugants'.

DNA is transmitted through conjugation

We used two different approaches to decide whether plasmid DNA can be transmitted from bacteria to yeast by a mechanism equivalent to bacterial conjugation: the first involved physically disturbing the conjugal environment so as to disrupt bacterial conjugation, and the second was to disrupt genetically the yeast-bacteria system and to determine whether transmission needed the plasmid loci necessary for conjugation between bacteria.

Physical requirements for DNA transmission. DNA transfer between bacteria by conjugation is distinguished from transduction or transformation by a dependence on cell contact, donor-cell viability and residence of the plasmid in the donor cell at the time of cell-to-cell contact. It was conceivable that the transmission of YEp13 to yeast occurs by a process unrelated to bacterial conjugation. For example, bacteria with conjugation plasmids may lyse more frequently or increase by other means the concentration of extracellular DNA in their vicinity. In four separate experiments we have demonstrated that the transmission of YEp13 DNA from bacteria to yeast has the same physical requirements as bacterial conjugation: (1) Exogenously

added plasmid is not transmitted to yeast cells. Yeast cells were subjected to the conjugation protocol in the presence of more YEp13 DNA than would be released if all bacterial cells in the bacteria-yeast conjugation system had lysed. The 'mock' conjugations were performed with YEp13 alone, with YEp13 and plasmid-free bacteria, or with YEp13 and *E. coli* containing only the pDPT51 plasmid. Leu⁺ yeast cells were not observed in any of these experimental combinations (Table 2*b*). The same yeast culture was mixed with *E. coli* cells containing both pDPT51 and YEp13 as a positive control, and Leu⁺ transconjugant yeast were evident. We also demonstrated that extracellular DNA could not be responsible for the occurrence of Leu⁺ yeast by adding DNaseI to the bacteria-yeast mixture, and we found no interference with DNA transmission (data not shown; ref. 13). (2) Viable donor cells are required. A culture of *E. coli* containing pDPT51 and YEp13 was split into three. The first sample was treated with chloroform to kill the cells, and the chloroform then removed¹⁴. The second sample was treated with buffer prewashed with chloroform in the same way as the first to test the efficiency of chloroform removal. The third sample was untreated. Leu⁺ yeast cells were not recovered when the chloroform-treated cells were used as donors, but were with the buffer-treated or untreated cells (Table 2*c*). (3) DNA transmission does not involve an extracellular diffusible factor. A

TABLE 3 Genetic requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Selection	Transconjugant cells per ml	Donor: recipient ratio	Transconjugant cells per donor
a1					
pDPT51 (<i>mob</i> -C, <i>mob</i> -R, <i>oriT</i> -R, <i>Tp</i> ^r)	Y	Leu ⁺	5 × 10 ³	1:1,000	7 × 10 ⁻²
YEp13 (<i>oriT</i> -C, <i>Tc</i> ^r , <i>LEU2</i>)	B	<i>Tp</i> ^r	4 × 10 ⁶	1:6,000	2
	B	<i>Tp</i> ^r + <i>Tc</i> ^r	5 × 10 ⁶	1:6,000	3
	B	<i>Tc</i> ^r	4 × 10 ⁶	1:6,000	2
a2					
R751 (<i>mob</i> -R, <i>oriT</i> -R, <i>Tp</i> ^r)	Y	Leu ⁺	0	1:100	<2 × 10 ⁻⁵
YEp13 (<i>oriT</i> -C, <i>Tc</i> ^r , <i>LEU2</i>)	B	<i>Tp</i> ^r	4 × 10 ⁶	1:4,000	2
	B	<i>Tp</i> ^r + <i>Tc</i> ^r	0	1:4,000	<2 × 10 ⁻⁶
	B	<i>Tc</i> ^r	0	1:4,000	<2 × 10 ⁻⁶
b1					
pDPT51 (<i>mob</i> -C, <i>mob</i> -R, <i>oriT</i> -R, <i>Tp</i> ^r)	Y	Ura ⁺	1 × 10 ³	5:1	3 × 10 ⁻⁵
YEp24 (<i>oriT</i> -C, <i>Tc</i> ^r , <i>URA3</i>)	Y	Leu ⁺	0	5:1	<3 × 10 ⁻⁷
pAL2 (<i>Cm</i> ^r , <i>LEU2</i>)	B	<i>Tp</i> ^r	2 × 10 ⁷	1:6	6
	B	<i>Tc</i> ^r	2 × 10 ⁶	1:6	4 × 10 ⁻¹
	B	<i>Cm</i> ^r	4 × 10 ⁴	1:6	8 × 10 ⁻³
b2					
pDPT51 (<i>mob</i> -C, <i>mob</i> -R, <i>oriT</i> -R, <i>Tp</i> ^r)	Y	Leu ⁺	1 × 10 ²	30:1	3 × 10 ⁻⁷
pAL2 (<i>Cm</i> ^r , <i>LEU2</i>)	B	<i>Tp</i> ^r	3 × 10 ⁵	3:1	3 × 10 ⁻¹
	B	<i>Cm</i> ^r	8 × 10 ⁷	3:1	3 × 10 ⁻³
b3					
pDPT51 (<i>mob</i> -C, <i>mob</i> -R, <i>oriT</i> -R, <i>Tp</i> ^r)	B	<i>Tp</i> ^r	2 × 10 ⁶	1:1	1 × 10 ⁻¹
pACYC184 (<i>Cm</i> ^r)	B	<i>Cm</i> ^r	0	1:1	<5 × 10 ⁻⁸
c1					
pFLEU2 (<i>mob</i> -F, <i>oriT</i> -F, <i>LEU2</i>)	Y	Leu ⁺	1 × 10 ²	5:1	5 × 10 ⁻⁸
c2					
pDPT51: <i>LEU2</i> (<i>mob</i> -R, <i>oriT</i> -R, <i>mob</i> -C, <i>LEU2</i>)	Y	Leu ⁺	1 × 10 ²	3:1	1 × 10 ⁻⁷
c3					
R751 (<i>mob</i> -R, <i>oriT</i> -R)	Y	Leu ⁺	0	6:1	<3 × 10 ⁻¹⁰
YEp13 (<i>oriT</i> -C, <i>Tc</i> ^r , <i>LEU2</i>)	B	<i>Tp</i> ^r	1 × 10 ⁸	2:1	5 × 10 ⁻¹
	B	<i>Tc</i> ^r	0	2:1	<3 × 10 ⁻⁹

Conjugation assays were performed as described for Table 2. During the course of these experiments it was discovered that a donor to recipient ratio of ~1:1,000 (10⁴–10⁵ donor cells per ml and 10⁷–10⁸ recipient cells per ml) resulted in a higher frequency of transmission than ratios closer to 1:1 (10⁸ cells per ml of both types). Experiments reported are representative, not an average of multiple experimental results. The *a* set of experiments demonstrated that plasmid transmission was dependent on *mob* activity. The donor bacterial strain was SB21, the recipient yeast strain was SY1229, and the recipient bacterial strain was RR1. Leu⁺ transconjugant yeast cells were selected by direct plating of the conjugation mixture on SD + his + ura (to select YEp13). Transconjugant bacteria were selected on OMBG + pro + leu + streptomycin (Sm; 100 µg ml⁻¹) medium which also contained either *Tp* alone (to select pDPT51 or R751) or both *Tp* and *Tc* (to select pDPT51 and YEp13). Transconjugant bacteria were also selected on LBH + Sm + *Tc* (to select YEp13). The *b* set of experiments established the specificity of the *mob*–*oriT* interaction. The donor bacterial strain was DH1 (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44*; ref. 32), the recipient bacterial strain was HB101 (*recA* derivative of RR1; ref. 25), and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by direct plating on SD + his + ura (to select pAL2) or on SD + his + leu (to select YEp24). Transconjugant bacteria were selected by plating on OMBG + pro + leu + Sm + *Tp* (to select pDPT51); on LBH + Sm + *Tc* (to select YEp24); or on LBH + Sm + chloramphenicol (*Cm*; 12 µg ml⁻¹) (to select pAL2 or pACYC184). Although there was no pAL2 transmission to yeast in our experiment *b1*, in repeat experiments it occurred at a frequency of 3 × 10⁻⁷. The *c* set of experiments demonstrated that derivatives of the F plasmid were capable of promoting DNA transfer to yeast. The donor bacterial strain was DH1 (*c1* and *c2*) or SB21 (*c3*), the recipient bacterial strain was HB101, and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by preincubation on solid non-selective medium for 5–12 h before plating on SD + his + ura (to select pFLEU2, pDPT51:*LEU2*, or YEp13). Transconjugant bacteria were selected by plating on OMBG + pro + leu + Sm + *Tp* (to select R751), or on LBH + Sm + ampicillin (*Am*; 100 µg ml⁻¹) (to select YEp13).

* Y indicates that the recipient organism was yeast; B indicates that the recipient organism was bacteria.

culture of *E. coli* containing pDPT51 and YEp13 was divided into supernatant and pellet fractions by filtration through a 0.45-µm pore Millipore filter and each fraction mixed with a yeast culture in a conjugation assay. Leu⁺ transconjugant yeast cells were recovered from the mixture of pellet and yeast, but no Leu⁺ transconjugants were recovered when the yeast cells were mixed with the supernatant fraction (Table 2d). (4) Cell contact is required for DNA transmission. Yeast and donor bacteria were applied to the surface of a 0.45-µm pore Millipore filter in two different orientations. In one orientation the yeast and bacteria were on the same side of the filter and in the other, the filter separated them. Leu⁺ transconjugant yeast cells were recovered only when the yeast and bacteria were on the same side of the filter (Table 2e).

Transconjugant yeast cells were recovered only when the conjugation plasmid and YEp13 were present in a viable donor and when cell contact was allowed, thus demonstrating that the requirements were the same as for bacterial conjugation.

Genetic requirements for DNA transmission. We investigated whether DNA transmission required the conjugation functions *mob* and *oriT*. If the transmission of DNA from bacteria to yeast used the *mob* and *oriT* functions required for bacterial conjugation, then the transmission process should discriminate between plasmids using the same rules for *mob*/*oriT* interaction that characterize bacterial conjugation.

To test whether the ColE1 *mob* functions were necessary for transmission of YEp13 from bacteria to yeast, we compared the ability of *E. coli* cells containing either pDPT51 or R751 to transmit YEp13 to yeast. As noted above, pDPT51 contains *mob*-C, whereas its parent, R751, does not (Table 1); also, pDPT51 promotes transfer of YEp13 to yeast whereas R751 does not (Table 3, *a1* and *a2*; Fig. 1). Matings of these donor bacteria with other bacteria verifies that the R751 and pDPT51 conjugation systems are operative and have the properties expected. As shown in Table 3, *a1* and *a2*, *E. coli* containing pDPT51 and YEp13 can transmit both plasmids, whereas *E. coli* contain-

ing R751 and YEp13 can transmit only R751 to the recipient bacteria. We conclude that transmission of plasmid DNA from bacteria to yeast requires *mob* functions that are compatible with the *oriT* on the yeast plasmid.

The importance of *oriT* in DNA transmission from bacteria to yeast was tested by comparing transmission of *oriT*-C⁺ and *oriT*-C⁻ plasmids co-resident in a bacterial strain. We constructed a bacterial strain carrying pDPT51 (to provide *mob*-C functions) as well as *oriT*-C⁺ or *oriT*-C⁻ yeast plasmids (YEp24 and pAL2, respectively). As shown in Table 3, b1, transmission of the *oriT*-C⁺ plasmid to yeast recipients is increased over that of the *oriT*-C⁻ plasmid by a factor of ~100. Bacterial matings gave the same relative ratio of transmission of the two plasmids. We did not expect pAL2 to exhibit any transmission to either yeast or bacteria because its parent plasmid, pACYC184, is strongly *oriT*-C⁻ and cannot be transmitted in bacterial matings (Table 3, b3; ref. 15). Evidently the *LEU2* or 2 μ sequence in pAL2 has weak *oriT*-C activity or can undergo site-specific recombination with a plasmid present in the bacterial donor. The salient observation is, however, that the *oriT*-C⁺ plasmid is transmitted at a much higher frequency than the *oriT*-C⁻ plasmid.

We then established that differences in plasmid copy number or competition for transfer machinery could not be responsible for the different transmission frequencies of YEp24 and pAL2. DNA was extracted from the donor cells and used to transform *E. coli*¹⁶. Chloramphenicol- (pAL2) and tetracycline-(YEp24) resistant transformants were obtained at nearly equal frequencies (data not shown). Also, a donor containing only pDPT51 and pAL2 transmitted pAL2 to yeast and bacteria at essentially the same frequency as the bacterial strain containing pDPT51, pAL2 and YEp24 (compare Table 3, b1 and b2). Thus, efficient transmission of a plasmid from bacteria to yeast seems to require that the plasmid have an *oriT* that can be acted on by *mob* gene products present in the donor cell.

F factor mediates conjugation with yeast

The experiments described so far rely on cell contact and transfer functions encoded by the broad-host-range plasmid R751 and its derivative pDPT51, both of which promote conjugation between *E. coli* and many other bacterial species. The F plasmid, however, confers a limited-host-range phenotype. We tested whether the apparent limited-host-range of such a plasmid could be extended to include yeast by the incorporation of a sequence which would allow it to replicate autonomously in yeast. This approach has already shown that derivatives of F can be transmitted to *Pseudomonas aeruginosa* when they contain a sequence that confers autonomous replication in that species⁸.

Plasmid pRS2405 (Table 1), a derivative of F (refs 9 and 10) that carries cell contact and transfer functions from F (*tra*-F)

as well as *mob*-F and *oriT*-F, was engineered to carry the yeast *LEU2* gene and the 2 μ replication sequence (pFLEU2; Table 1). To compare directly the transmission of the F plasmid to the R751-based plasmids, we constructed a derivative of pDPT51, pDPT51:LEU2, that could be monitored in yeast by virtue of *LEU2* and 2 μ sequences. *E. coli* cells containing one or the other plasmid were able to conjugate with yeast to yield Leu⁺ transconjugants (Table 3, c1 and c2), although transmission was less than that measured for YEp13 co-resident with pDPT51 (Table 3, a1). Both pFLEU2 and pDPT51:LEU2 (>50 kilobase pairs (kb)) are larger than YEp13 (11 kb) and so could either be transmitted less efficiently or have more difficulty in becoming established in yeast. Whatever the explanation, transmission of pFLEU2 and pDPT51:LEU2 reflects conjugal transmission to yeast, because we detected no transconjugant yeast cells in a control cross involving donor cells which could not transmit YEp13 (Table 3, c3).

Conclusions

We have found that bacterial plasmids that mediate conjugation between bacterial cells are also able to mediate plasmid transmission to the yeast *S. cerevisiae* by a process that seems, by our physical and genetic criteria, to be conjugation. Of the genetic functions required for conjugation between bacteria, we have shown that *mob* and *oriT* are necessary for bacteria-yeast conjugation. It remains to be determined whether *tra* functions required for cell-surface interactions in bacterial conjugation also participate in bacteria-yeast conjugation. Nonetheless, it is clear that two distinct cell-contact and transfer functions, one encoded by R751 and the other by F, and two *mob/oriT* systems, *mob*-C/*oriT*-C and *mob*-F/*oriT*-F, are capable of promoting DNA transmission from bacteria to yeast.

The conjugation functions encoded by the conjugative plasmids can apparently form contacts competent for DNA transfer with organisms separated by large evolutionary distances. Our results suggest that conjugation could occur between bacteria and animal cells and between bacteria and plant cells, even when the bacteria do not carry the specialised tumour-inducing plasmid. The conjugation system we have studied here provides a mechanism for the transfer of DNA between distantly related species. Such lateral transmission could be a significant factor in evolution and may help to explain discrepancies in phylogenetic trees^{17,18}.

Finally, our system provides a rapid method, which in some cases could be superior to DNA transformation, for introducing plasmids into yeast and possibly into other organisms. Moreover, the system may provide a tractable way to investigate the molecular mechanism of transfer of DNA from *A. tumefaciens* to plant cells should it be possible to substitute yeast for the plant component. □

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Demonstration of the phase coherence of the superconducting wavefunctions between conventional and high- T_c superconductors

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IF phase coherence of the quantum-mechanical wavefunctions between conventional (BCS) and high-transition-temperature (high- T_c) superconductors were shown to exist, this would place powerful constraints on the as yet unknown mechanism in the latter materials. Here we demonstrate the quantum coherence of the macroscopic superconducting wavefunctions of a conventional and a high- T_c superconductor by the observation of persistent supercurrents and the quantization of magnetic flux in units of $h/2e$ within a composite ring of niobium and $\text{YBa}_2\text{Cu}_3\text{O}_7$ (YBCO). This shows unambiguously that the macroscopic wavefunctions are coupled at the interface between the two superconductors, regardless of the mechanism and nature of electronic pairing in high- T_c superconductors. The experiment is a variant of an earlier measurement in which the quantization of flux was measured in an YBCO superconductor ring¹. In our experiment, an adjustable niobium point was used to bridge the gap in a superconducting circuit of YBCO. In addition to observing persistent supercurrents around the ring with discrete flux states separated by the flux quantum, $h/2e$, the critical current across the junctions also exhibits the expected flux-quantum periodicity. This provides the first demonstration of a d.c. superconducting quantum interference device (SQUID) formed from a composite high- T_c /BCS superconducting circuit.

There remains considerable controversy and conflicting experimental data concerning the microscopic mechanism for superconductivity in high- T_c superconductors and the resulting nature of the electron pairing (ref. 2 and references therein). In a conventional BCS superconductor the electrons are paired in s -states with no net spin or orbital motion. Other mechanisms for pairing, such as might exist in high- T_c superconductors, could lead to a macroscopic superconducting wavefunction with p - or d -type symmetry similar to the superfluid phases of liquid ^3He . For example, the resonant-valence-bond (RVB) model³ for high- T_c superconductors has been predicted to lead to d -wave superconductivity⁴. Any experiment that relates to the nature of the electron-pair wavefunction in high- T_c superconductors is therefore potentially of fundamental importance.

The symmetry of the superconducting wavefunctions might be expected to affect the quantum tunnelling of superconducting electrons across an interface between a conventional and high- T_c superconductor. The presence of Josephson tunnelling across a boundary between an s - and a p - or d -like superconductor may provide valuable information about the nature of the p - or d -wavefunctions⁵. In practice, Josephson tunnelling has been observed across an interface between a conventional and high- T_c superconductor^{6,7}. However, there are problems in the interpretation of such measurements. Indeed, apparent Josephson-junction tunnelling between a normal metal and a lanthanum-based high- T_c superconductor has been observed⁸. In this case, as Josephson tunnelling cannot occur between a normal metal-superconductor interface, the weak links responsible for the Josephson characteristics had therefore to be within the high- T_c material and not at the interface. In contrast, Josephson coupling between a niobium/YBCO interface has been inferred from the temperature dependence of the Josephson current⁶.

To provide an unambiguous demonstration of quantum phase coherence across an interface between a conventional superconductor and a high- T_c superconductor, our experiment was designed to verify flux quantization within a composite high- T_c /BCS superconducting ring. The measurements were made using an incomplete high- T_c superconducting ring with the gap in the high- T_c ring bridged by a conventional superconductor.

The high- T_c material was a 13-mm diameter, 3-mm-thick disk of single-phase $\text{YBa}_2\text{Cu}_3\text{O}_7$. Two holes of diameter 2.5 mm and 2.1 mm were drilled through the disk with 0.5-mm slots machined between them, and also to the outer perimeter of the disk (Fig. 1). A 60° niobium tip was screwed down into the slot at a position close to the perimeter of the disc to form a continuous superconducting circuit including the two holes. The strength of the two 'bridging contacts' formed between the sloping sides of the niobium tip and the upper edges of the YBCO slot could easily be adjusted by a mechanical screw operated at room temperature. A toroidal coil passed through hole A to provide a known amount of external flux within the completed superconducting loop. The second hole, B, contained the primary coil of a d.c. superconducting flux transformer with the secondary coil weakly coupled to a conventional r.f. SQUID to monitor changes in flux within the superconducting loop. Electrical leads were attached to the niobium and YBCO superconductors so that the V - I characteristics across the niobium/YBCO contacts could also be measured.

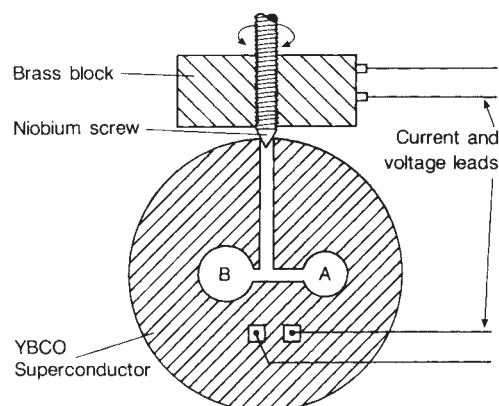


FIG. 1 Schematic diagram of the composite ring. The niobium point, when in contact with both sides of the slot, forms a complete superconducting circuit.

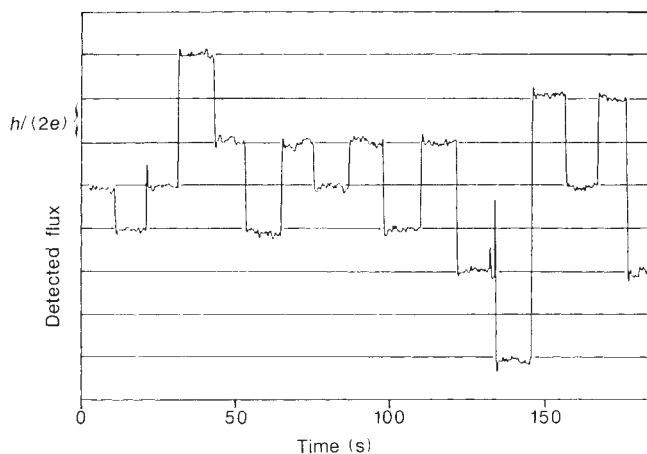


FIG. 2 Flux within the hybrid Nb/YBCO superconducting ring as a function of time. Periodic bursts of electromagnetic radiation cause transitions between otherwise-stable quantum states. The measurements were made at 4.2 K.

The niobium point was screwed down to bridge the slot in the YBCO disk at liquid-helium temperatures and all measurements reported here were made at 4.2 K. The superconducting contacts were remarkably stable to thermal cycling within the liquid-helium temperature range and could easily be adjusted to the desired strengths. No attempt has been made to test thermal recyclability from liquid-helium to room temperature, but such recyclability is unlikely to occur in view of the difference in thermal-expansion coefficients of the two superconductors.

Before the contacts were made, there was almost no magnetic coupling between the toroidal coil and the flux transformer. On closing the superconducting loop, however, the flux measured by the detecting circuit was directly proportional to the flux applied by the toroidal coil, which follows from the conservation of total flux within a superconducting circuit. Such measurements provide a direct calibration of the flux sensitivity of the SQUID detection system in terms of an exactly known flux within the completed superconducting loop. On increasing the applied flux, the induced supercurrent eventually exceeds the critical current of the weakest junction in the circuit and flux can move in or out of the ring. The induced supercurrents showed no indication of decay on the timescale of a measurement (typically 20 min), implying a phase coherence around a fully superconducting circuit or a resistance at the interface of $R \ll 10^{-13} \Omega$.

As a definitive test of macroscopic quantum coherence, transitions between the quantum states of the ring were induced by periodically exposing the ring to a short burst of 5-MHz radiation. These transitions, corresponding to discrete changes of flux within the ring in multiples of the flux quantum, are illus-

trated in Fig. 2. The horizontal lines denote the expected separation of flux states assuming that flux is quantized in units of $h/2e$. The observation of discrete changes of flux in multiples of $h/2e$ proves that the superconducting wavefunctions of the high- T_c and conventional superconductors are coupled at their interface, allowing a coherent superconducting wavefunction to persist around the composite high- T_c /BCS superconductor ring.

As another demonstration of quantum coherence, a modified geometry was used which enabled us to devise a d.c. SQUID, based on the quantum interference of the superconducting wavefunctions, closely analogous to the double-slit diffraction experiment in optics⁹. In this geometry, a single slot between the two holes drilled in the YBCO disk was used, with no slot to the perimeter. The niobium point was then used to bridge the slot connecting the two holes, resulting in two superconducting loops around the holes in the YBCO having a common niobium section with two niobium/YBCO interfaces.

An electrical current was passed from the niobium to the YBCO and the voltage across the structure was recorded in a standard four-terminal configuration. Figure 3a shows a typical set of V - I characteristics.

Quantum coherence around the hybrid superconducting circuit was also demonstrated in this geometry. We monitored the flux in one hole as the flux in the second hole was varied; in the absence of the niobium bridging contact, the flux in the second hole is directly proportional to the externally applied flux, but, on bridging the connecting slot with weakly coupled Nb/YBCO contacts, the flux becomes periodic in the flux quantum, as shown in Fig. 3b.

We demonstrated quantum interference by adjusting the point contacts until the critical current determined from the V - I characteristics exhibited periodicity with applied flux. We adjusted the current to just above the critical current (point A on Fig. 3a) and observed the change in voltage as the applied flux was varied (Fig. 3c). All the measurements shown in Figs. 3a-c are for the same setting of the niobium point.

The periodicity in the critical current arises from the quantum interference of the superconducting wavefunctions that describe the supercurrents which divide between the two Nb/YBCO junctions on passing from the niobium to the YBCO (see ref. 9 for an account of quantum interference effects in superconducting circuits). This is the first demonstration of a d.c. SQUID using a bulk high- T_c superconductor with adjustable point-contact junctions.

Our experiments confirm the coherence of conventional BCS and high- T_c superconductor wavefunctions around a hybrid circuit containing the two types of superconductor. If the coupling mechanism at the interface of the two superconductors preserves the symmetry of the wavefunction, the above experiment shows that high- T_c superconductors is s -like, as in conventional superconductors. However, if the interface involves a symmetry-breaking mechanism, such as spin-orbit coupling, the possibility of p - or d -type superconductivity for high- T_c materials cannot be definitely excluded. Indeed, coupling between s - and d -wavefunctions will be produced by any rotational symmetry-breaking interaction, which inevitably occurs at an interface⁵. □

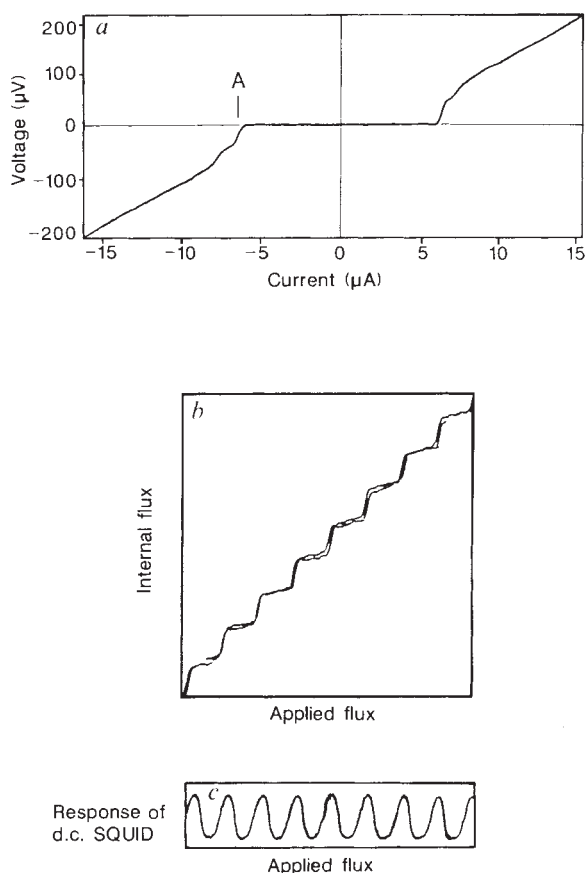


FIG. 3 Measurements on a two-hole d.c. SQUID; a, V - I characteristic. b, Measured flux as a function of applied flux. c, Periodic variation in voltage across the SQUID as a function of the same applied flux.

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Dipole formation and collisions in a stratified fluid

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BECAUSE of its relevance to large-scale geophysical flows, two-dimensional turbulence has attracted increasing attention during the past two decades. An important feature of such flows is the so-called inverse energy cascade—the spectral flux of kinetic energy from small to larger scales of motion^{1,2}. Numerical simulations of two-dimensional turbulence have demonstrated the emergence of coherent vortices from a randomly generated initial flow field^{3,4}. These calculations revealed the occurrence of two categories of coherent structures, specifically, the monopolar axisymmetric vortex, characterized by a non-zero angular momentum, and the dipolar vortex, characterized by a non-zero linear momentum and a steady translation; recent experiments^{5,6} demonstrated the existence of a tripolar vortex structure, characterized by a non-zero angular momentum and a steady rotation of its axis. Here we report on the formation of two-dimensional dipole structures by the gravitational collapse of a compact region of three-dimensionally turbulent, mixed fluid in a quiescent stratified environment. The dipole thus produced seems to be very stable, its robustness being demonstrated by experiments on colliding dipoles.

The experiments were conducted in a square perspex tank of horizontal dimensions 1×1 m and a working depth of 0.3 m, which was filled with a linearly stratified salt solution. A fixed volume of fluid was injected horizontally through a nozzle with a small diameter d . The injection velocity U was typically 2 m s^{-1} , the nozzle diameter $d \approx 10^{-3} \text{ m}$ and the kinematic fluid viscosity $\nu \approx 10^{-6} \text{ m}^2 \text{ s}^{-1}$, so that the Reynolds number, based on the nozzle diameter, had a characteristic value greater than 2×10^3 , which ensured that the outflow was turbulent. Care was taken to ensure that the density of the injected fluid exactly matched the density of the ambient stratified fluid at the level of the nozzle, so as to create a purely horizontal jet. Because the fluid was injected during a short period of time (typically 1 s). The effect of the ambient density stratification was negligible during this period, and turbulent entrainment occurred as in a non-stratified fluid, resulting in a cone-shaped turbulent region.

The flow was visualized by adding dye to the injected fluid, and the subsequent distortion of the region of dyed fluid was recorded both from above and from the side by remote-controlled photographic cameras. These observations revealed that gravitational collapse of the mixed region started soon after the injection forcing had stopped. During this collapse the vertical velocity diminished quickly, and the dyed fluid soon occupied a flat, horizontal region in which the motion was almost purely horizontal. At this stage the horizontal eddies were observed to merge and increase in size, and eventually the flow became organized into a large dipole. Figure 1 shows this sequence of events during dipole formation, as photographed from above. Once the dipole was formed it moved horizontally along a straight line, without any appreciable changes in its shape. Under the present experimental conditions it occupied a pancake-like region of typically 2–4 cm in thickness and 15–20 cm in width, and its translation speed was $\sim 10^{-3} \text{ m s}^{-1}$. The Reynolds number of this flow structure, based on its diameter, is typically 10^3 . Qualitative information about the vertical distribution of horizontal velocities in the dipole region was obtained by dropping a few KMnO_4 crystals into the tank. The distortion of their vertical dye trails revealed that the motion inside the dipole was, to a good approximation, two-dimensional, except in two relatively thin (viscous) transition layers on the upper and lower faces of the dyed region. The spiral-shaped dye

distributions in the developed dipole (Fig. 1d) indicate that undyed ambient fluid is entrained at its rear. It is probably this lateral entrainment of initially quiescent fluid, as well as the presence of horizontal viscous shear layers on the upper and lower faces of the mixed region, that cause the observed gradual decrease of the dipole's translational speed.

During the collapse of the turbulent mixed region and the subsequent dipole formation, internal waves are generated, by means of which energy is radiated away from the centre of the collapsed region. Although they get reflected by the lateral walls of the tank, these waves were observed to decay fairly quickly and clearly did not affect the dipole structure.

Quantitative information about the flow field was obtained by streak photography of neutrally buoyant tracer particles that were illuminated from the side by a slit light. Measurement of the streak lengths allows an accurate reconstruction of the horizontal velocity field, from which other flow characteristics such as the vorticity ω and the stream function Ψ can be calculated numerically. Analytical solutions for dipolar vortex structures (for example, Lamb's dipole⁷ and Stern's modon⁸) have been obtained by assuming a linear relationship between those quantities. Recent experiments⁹ and numerical simulations⁵, however, suggest that 'realistic' dipoles are characterized by a nonlinear relation between ω and Ψ . This is confirmed by the present experiment (Fig. 2). Further work along these lines is still in progress, and it is anticipated that detailed information about the (ω, Ψ) relation may lead to important refinements of the existing dipole models.

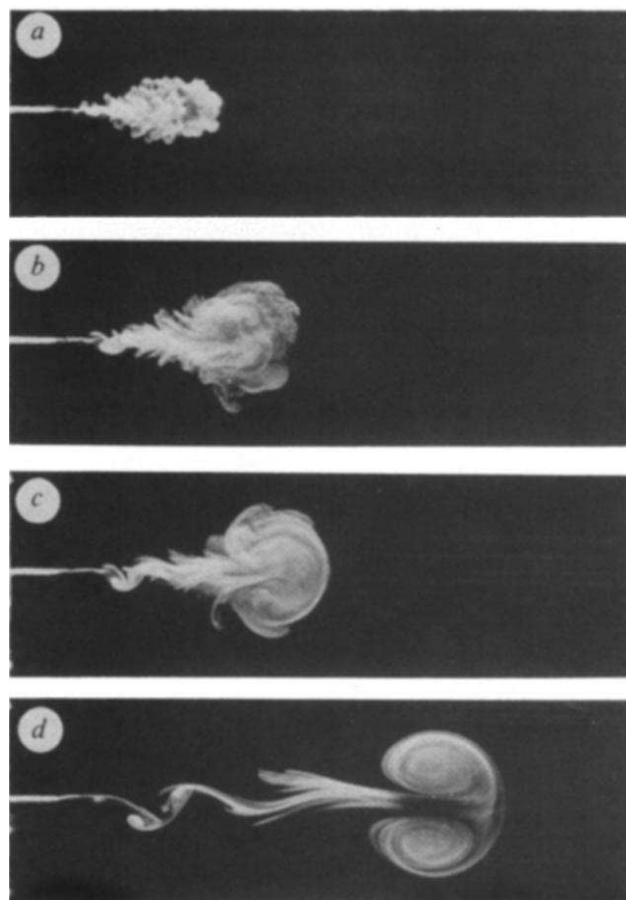


FIG. 1 A sequence of plan-view photographs illustrating the emergence of a dipolar vortex from a horizontally injected fluid in a stratified environment. The pictures were taken *a*, 3 s, *b*, 17 s, *c*, 38 s and *d*, 240 s after the injection was stopped. Experimental parameters are: injected volume $Q = 3.66 \text{ cm}^3$, duration of injection $\Delta t = 0.6 \text{ s}$, nozzle diameter $d = 1.9 \text{ mm}$, and buoyancy frequency of the ambient stratification $N = 1.68 \text{ s}^{-1}$.

The robustness of the dipolar vortex structure was studied in further experiments on frontally colliding dipoles. Fluid was injected simultaneously from two nozzles placed opposite each other at some distance apart. In the first experiment the jets were created under identical conditions, ensuring that both dipoles had approximately identical characteristics. The injected fluids were dyed with different colours, which allowed observation of any mass exchange during the collision. The sequence of events during the interaction is illustrated by the plan-view photographs in Fig. 3. Although the colliding dipoles were not exactly symmetric and were slightly mis-aligned (Fig. 3*b*), it is clear (and more so in the original colour photographs) that the dipoles exchange partners (Fig. 3*c*), and that two new dipoles arise, moving along straight lines away from the collision area (Fig. 3*d*). This new axis of motion is not exactly perpendicular to the original axis, but this is attributed to the mis-alignment of the original vortices. It can be inferred from the distribution of the dyes (visible in Fig. 3 as different grey tones) that no mass is exchanged between the partners: except for some 'streamers' wrapped around the respective new partners—an effect again attributed to the initial mis-alignment—each partner appears to conserve its own mass.

In the second experiment on dipole interactions, a centred head-on collision was created between two dipoles of slightly different size and strength. Plan-view photographs showing the sequence of events during the collision process are shown in Fig. 4. As in the previous collision experiment, the dipoles exchange partners (without any mass transfer between them) and the newly formed dipoles move away from the collision region (Fig. 4*c*). In contrast to the previous experiment, however, the newly formed dipoles are not symmetric (because of the

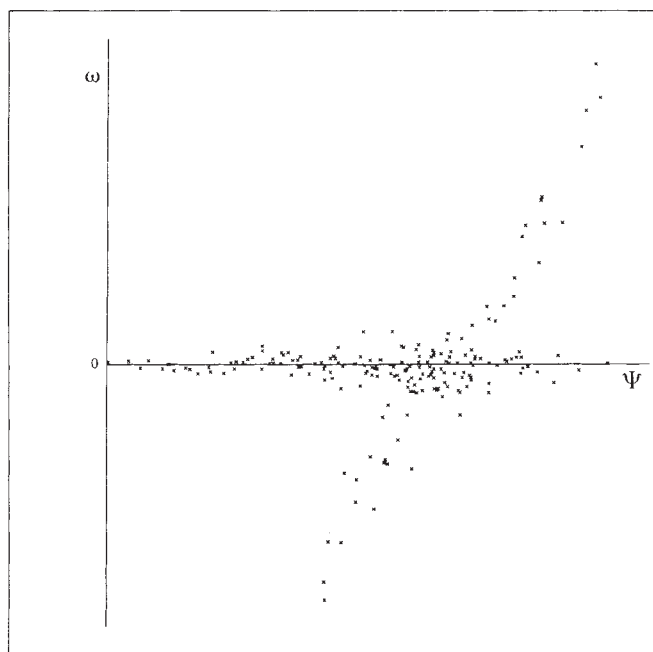


FIG. 2 A typical scatter plot showing the relation between the vorticity ω and the stream function Ψ in the flow field associated with a dipolar vortex. After digitizing the streak-lines, the velocity is calculated on a regular grid by numerical interpolation. For each grid point, ω and Ψ are then calculated from this interpolated velocity field, and each set of (ω, Ψ) values thus obtained provides a point in the scatter plot. A correction has been made for the translation of the dipole structure, whose speed is measured from photographs taken at subsequent times. The band of zero vorticity represents the region outside the dipole, whereas the branches of positive and negative vorticity correspond to the dipolar vortex. The nonlinearity of the (ω, Ψ) relation within the dipole is evident.

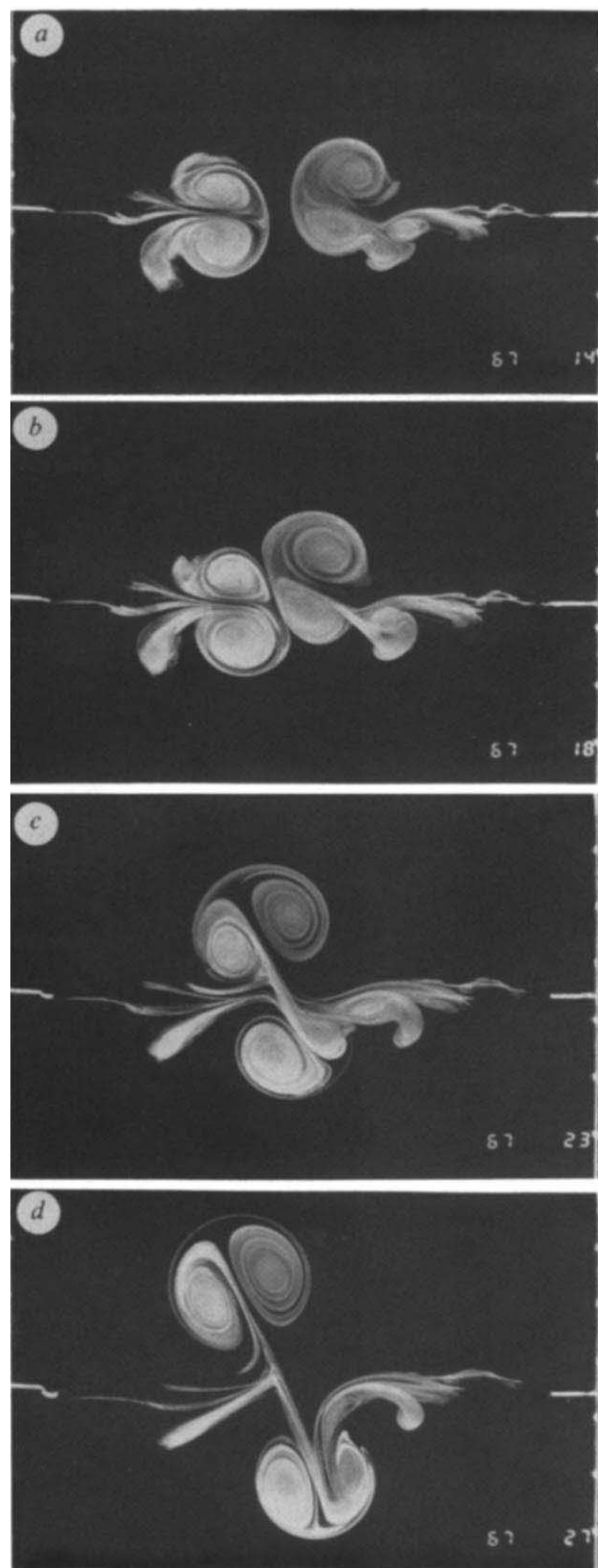


FIG. 3 A sequence of plan-view photographs showing consecutive stages of the head-on collision of two dipoles. The original dipoles were dyed with different colours, evident here as different grey tones. The photographs were taken *a*, 42 s, *b*, 70 s, *c*, 120 s, and *d*, 225 s after stopping the injections. Experimental parameters (see Fig. 1 legend for explanation): $Q = 3.66 \text{ cm}^3$, $\Delta t = 0.57 \text{ s}$, $N = 2.25 \text{ s}^{-1}$ (Q and Δt were equal for both jets).

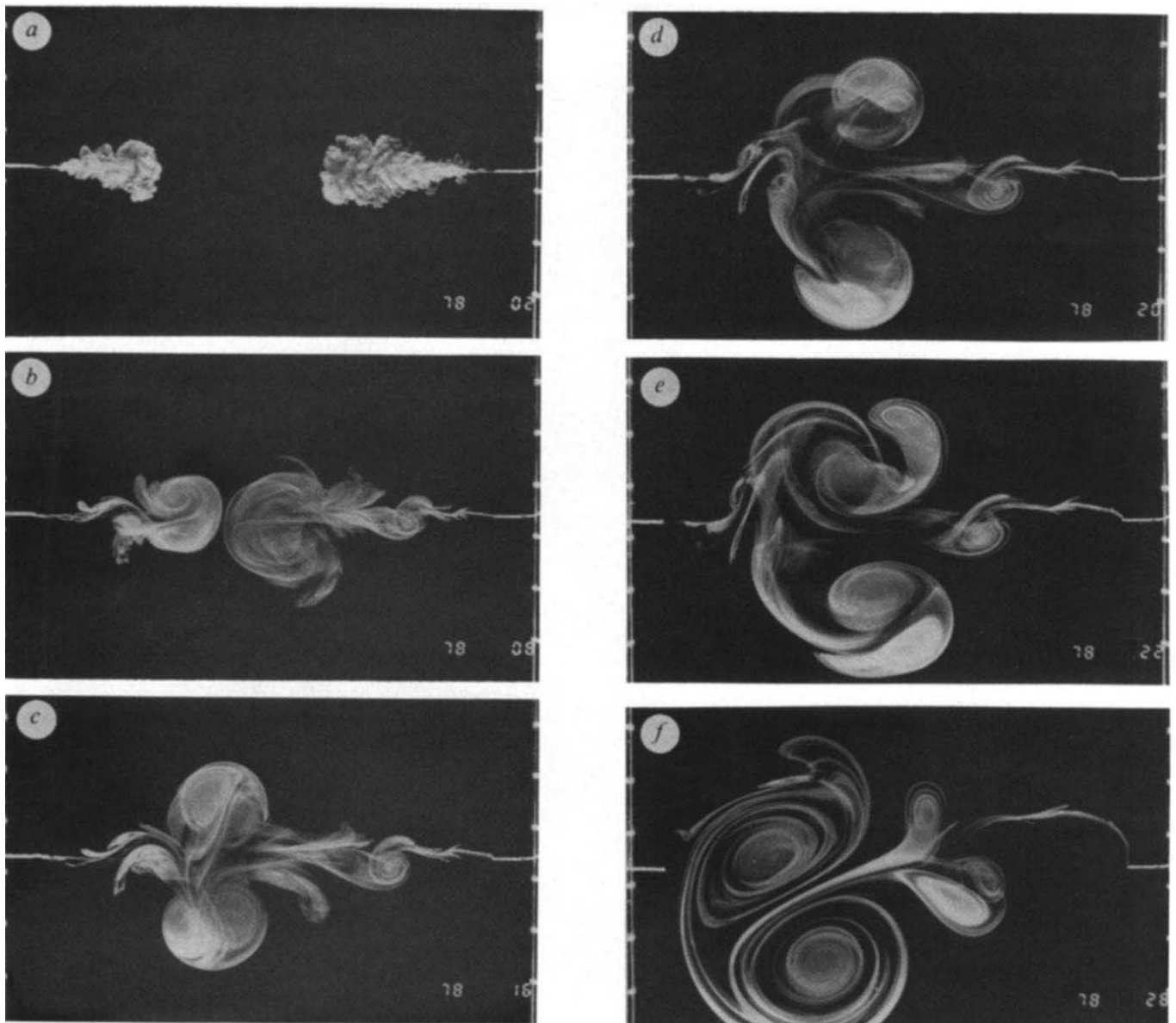


FIG. 4 Same as Fig. 3, but with dipoles of different strengths. The pictures were taken *a*, 4 s, *b*, 32 s, *c*, 67 s, *d*, 134 s, *e*, 224 s and *f*, 805 s after stopping the injections. Experimental parameters (see Fig. 1 legend for

explanation): $Q = 4.88 \text{ cm}^3$ and 2.44 cm^3 for the strong and the weak jet, respectively, with $\Delta t = 0.4 \text{ s}$ and $N = 1.9 \text{ s}^{-1}$.

different sizes of the original dipoles), and as a consequence they perform looping excursions and collide again at the original axis (Fig. 4*d, e*). Had these dipoles contained enough momentum at this stage, this second collision would have resulted in another exchange of partners, reforming the original dipoles which would have subsequently separated by propagating in opposite directions along the original axis. In these experiments, however, the dipoles were unfortunately too weak to follow this sequence, and ultimately only the large dipole survived, the smaller one being left behind and seriously deformed (Fig. 4*f*). Nevertheless, these collision experiments are generally consistent with the results of numerical simulations of interacting coherent vortex structures¹⁰⁻¹³, in the sense that they show the characteristic exchange of partners, the formation of new dipoles and the looping trajectories of these secondary dipoles in the case of colliding dipoles of different strengths.

In these experiments, two-dimensionality of the flow was established by the density stratification of the ambient fluid.

Two-dimensional flows can also be achieved in different ways, for example by a background rotation of the fluid system¹⁴, by magnetic forces⁹ or by confining the fluid in an essentially two-dimensional configuration such as a soap film¹³, and the emergence of dipolar (and other) coherent flow structures has also been observed in these situations. In contrast to the previous laboratory experiments on colliding dipoles^{9,13}, however, our experiments offer the unique possibility of studying mass transfer between these coherent vortex structures during mutual interactions. □

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Sediment supply and the development of the coarse surface layer in gravel-bedded rivers

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THE bed surface of most gravel rivers is considerably coarser than the sub-surface or the gravel load transported over it, a phenomenon affecting river dynamics, morphology and ecology. The coarse surface layer, often called an armour or pavement, has been attributed to an inherent tendency for small grains to settle between larger ones during active transport of all sizes^{1,2}; and to selective erosion or trapping of finer particles when the coarse grains are relatively immobile^{3,4}. Where bedload supply is cut off below dams, selective erosion causes coarsening^{5,6}. Using a simple quantitative model, we propose that surface coarsening develops in gravel-bedded rivers when local bedload supply from upstream is less than the ability of the flow to transport that load. We present laboratory results which support this hypothesis, and show that supply reduction causes changes in bedforms and progressive confinement of active bedload transport to a narrow band of finer bed surface bordered by a coarse, less active bed. It may therefore be possible to relate the degree of river-bed surface coarsening to sediment supply resulting from land use.

In rivers, when the boundary shear stress τ_b imposed by the flow exceeds the critical boundary shear stress τ_c required to mobilize grains on the stream bed, the bedload transport rate q_b is observed to increase rapidly with small increases in τ_b . The simplest approximate equation for predicting q_b is

$$q_b = k(\tau_b - \tau_c)^n \quad (1)$$

where k and n are empirically determined (n is usually close to 1.5)⁷. On a bed composed of many different grain sizes, τ_c is roughly proportional to the median grain size of the bed surface⁸. Flume and field studies indicate that a stream bed's sub-surface is similar in grain size distribution to the stream's bedload⁹. We propose that the disparity between median grain size of the surface and that of the sub-surface or load can arise when the transport rate exceeds the local supply rate. This imbalance may cause net erosion, but in poorly sorted sediment (that is, sediment with a wide grain size distribution), it may also cause selective erosion and deposition and vertical winnowing to coarsen the bed and raise the critical shear stress. Our model differs from those that argue that armouring is inherent in the transport of poorly sorted sediment^{1,2}; we propose instead that surface coarsening occurs primarily where there is a transport-supply imbalance, and it may occur over small parts of the stream bed or over the entire stream bed.

Using equation (1), we can form a dimensionless sediment transport ratio q_* , which is the transport rate for the coarse surface normalized by the transport rate for a surface as fine as

the sub-surface or load:

$$q_* = \left(\frac{\tau_b - \tau_{cs}}{\tau_b - \tau_{cl}} \right)^{1.5} = \left(\frac{\tau_b - \alpha \left(\frac{D_{50_s}}{D_{50_l}} \right)}{\tau_b - 1} \right)^{1.5} \quad (2)$$

where τ_{cs} and τ_{cl} are the critical boundary shear stresses of the surface and the sub-surface or load, respectively, the parameter α for gravel with uniform specific gravity is unity, and D_{50_s} and D_{50_l} are the median grain size of the surface and load, respectively. An alternative⁷ to equation (1) can be manipulated to give

$$q_* = \frac{\left[\frac{\tau_b}{\tau_{cs}} - 1 \right] - \frac{1}{a} \ln \left(1 + a \left[\frac{\tau_b}{\tau_{cs}} - 1 \right] \right)}{\left[\frac{\tau_b}{\tau_{cl}} - 1 \right] - \frac{1}{a} \ln \left(1 + a \left[\frac{\tau_b}{\tau_{cl}} - 1 \right] \right)} \quad (3)$$

where the bracketed expressions represent the dimensionless excess shear stress for the median grain size of the surface and the load. Our hypothesis implies that q_* in equations (2) or (3) should be unity when sediment supply rate matches the river's ability to transport the load, and should decrease towards zero as the surface coarsens when supply is reduced.

We progressively reduced the sediment supply to a small flume, holding the water discharge and the bedload grain size distribution constant. Sediment identical to the supply was placed on the flume bed while damp to minimize settling of finer sediment between the larger grains. Nonetheless, simple wetting of the surface and grain vibrations (produced by water flow rates below that required for sediment transport) coarsened the bed significantly (Fig. 1) as the finer grains infiltrated the bed. As the surface reached equilibrium at a high bedload transport rate (17.4 g min⁻¹ per cm width), it became distinctly finer and more poorly sorted; however, the median grain diameter, or D_{50} , of the bed surface was equal to that of the load (3.7 mm) within measurement error. Reducing the sediment supply rate to 6.1 g min⁻¹ per cm width coarsened the surface to $D_{50} = 4.3$ mm, with a marked reduction in bed surface sand. Decreasing the sediment loading to 1.7 g min⁻¹ per cm width coarsened the surface further ($D_{50} = 4.9$ mm).

The bed surface showed spatial segregation of grain sizes into distinct zones which varied with supply (Fig. 2). At the high transport rate, the surface was covered with mobile, thin (1–2 grain diameters) bedload sheets¹⁰, consisting of alternating congested (coarse), smooth (fine), and transitional (intermediate) zones^{11,12}, migrating downstream (in that order), thus creating strong pulsations in bedload delivery to the flume outlet. As the sediment supply rate was reduced, sheets became less frequent and distinct, coarse inactive zones formed and expanded, and sediment transport became confined to a progressively narrower fine-textured active zone (Fig. 2) where bedload travelled in indistinct long-wavelength pulses.

The constriction of active bedload zones with decreasing supply probably results from interactions between coarse and fine grains. As the bed coarsens because of diminished supply, the finer bedload will tend to stop in the pockets formed by the coarser surface particles. Locally, if a sufficient number of fine particles stop, the pockets will partially fill, causing a reduction in local grain friction and a reduction in the fluid momentum loss from particle wakes. These effects will cause this local area to transmit more load and to have a higher flow velocity (causing locally convergent near-bed flow¹³), leading to an active bedload zone bounded by less active coarse surfaces.

Values of q_* predicted by equation (3) compare well with our experimental results (Fig. 3), supporting the simple explanation for surface coarsening proposed here. We used equation (3) rather than (2) for our own data and that of Parker *et al.*⁹ because the Yalin bedload equation predicted the observed transport rates more accurately. We estimated τ_{cs} and τ_{cl} in

equation (3) by using the relationship $\tau_{*c} = \tau_c \times [(\rho_s - \rho) \times g D_{50}]^{-1} \approx 0.045$ (where ρ_s and ρ are the grain and fluid density, respectively, and g is gravitational acceleration), recently proposed^{15,16} for poorly sorted coarse sediments. We calculated a in equation (3) as $a = 1.66 \tau_{*c}^{0.5} = 0.35$. The boundary shear stress was calculated from flow depth, water surface slope, and bed surface slope; it decreased (from 52 to 47 and then to 39 dyne cm^{-2}) with decreasing surface slope as the sediment supply was reduced between the three runs. The uncertainty in calculated τ_b (because of variability of bed slope over time) was close to the differences between these runs, however, and the family of curves shown in Fig. 3 all exhibit a similar rapid decline in q_* . Two data points calculated from experiments by Parker *et al.*⁹ under similar conditions are shown (Fig. 3); their τ_b/τ_{cl} ratio was ~ 2.5 . The q_* values for these two points were calculated from the observed initial and final bedload discharge rate in run 4 and from the estimated initial and observed final bedload discharge in run 2. For the Kuhnle and Southard¹⁴ data, q_* was calculated in two ways: by assuming that the highest feed rate (run H5) defined the transport rate without a coarse surface layer, and by calculating this transport rate for the same $\tau_b/\tau_{cl} \approx 4.2$ as by other runs. The predicted relationship is from equation (2) rather than from (3) because the bedload equation behind equation (2) gave a better estimate of observed sediment transport rates. Because the experiments of ref. 14 used supercritical flow, and the water surface slopes and equilibrium transport for all grain sizes were not reported, the quantitative inter-

pretation of their results is uncertain. We assumed that their bed slope and average flow depth could be used to estimate the average boundary shear stress using the Williams^{17,20} side-wall correction.

Parker and Klingeman² argued that the coarse surface layer developed by vertical winnowing of fines, making coarser grains more abundant at the surface and giving the coarse and fine fractions of the load equal mobility. They proposed that a coarse surface layer was generally required to transmit poorly sorted coarse bedload when boundary shear stresses were not far above critical. Our analysis suggests that their experiments were conducted under very low sediment feed rates, relative to the boundary shear stress imposed. This imbalance between sediment supply and transport led to surface coarsening which reduced the difference between the imposed boundary shear stress and the critical shear stress. Kuhnle and Southard¹⁴ varied sediment feed and water discharge in experiments which they interpret as supporting the Parker-Klingeman equal-mobility hypothesis. They imposed large initial excess boundary shear stresses ($\tau_b/\tau_{cl} > 4$) relative to the imposed load, however, and consequently a coarse surface layer developed in a manner consistent with our theory (Fig. 3). Although they conclude that their results support the equal-mobility hypothesis, they also propose that the initial surface in their experiments was too fine to accomplish equal mobility for the imposed flow and sediment feed rate. Hence, indirectly, they recognized the importance of sediment supply in their experiments.

We propose that surveys of rivers to determine patterns of q_* may provide a quantitative method to determine the sensitivity of rivers to sediment supply changes resulting from land use. Such a tool would be useful where conflicts arise between land use (such as forest cutting) and river use (such as fish production). Spatial variation in bed surface texture can be related to channel topography through detailed mapping of rivers at low flow. The local dimensionless transport rate q_* can then be estimated directly from τ_b (from the bankfull channel geometry), τ_{cs} (from bed-surface grain size analysis) and τ_{cl} (from sampling of the bed sub-surface, whose grain size distribution approximately equals the load⁹) for different grain-size zones. Such field surveys of rivers in California have revealed significant q_* variation with sediment supply similar to that predicted by our theory (D. Kinerson and W. E. Dietrich, manuscript in preparation).

We suggest that, in rivers with predominantly low q_* values, increased bedload supply as a result of accelerated erosion may be primarily accommodated through a local increase in q_* values

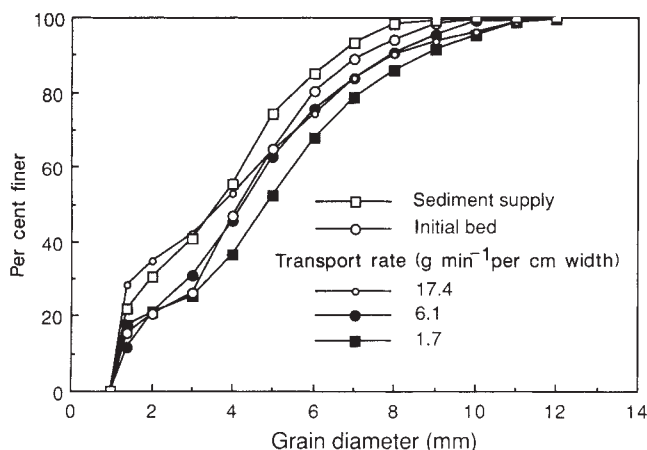


FIG. 1 Changes in bed-surface grain size distribution with sediment supply reduction. Cumulative size distributions shown for grain mixture fed into the flume (sediment supply), initial bed surface after wetting but before any sediment transport (initial bed), and bed surfaces under bedload transport rates of 17.4, 6.1, and 1.7 g min^{-1} per cm width.

METHODS. The flume was 7.5 m long, 0.3 m wide, and was kept at a constant slope of 0.0046. Water surface slope, gravel bed slope, and bed surface texture were free to equilibrate at the imposed rates of water discharge ($0.61 \text{ s}^{-1} \text{ cm}^{-1}$) and sediment loading (varied between runs as above). The highest sediment loading rate was adjusted to the observed bedload discharge from the initial bed. Fresh sediment was fed constantly into the upstream end of the flume by hand or conveyor belt. Discharged bedload was collected from the flume outlet at 5 min intervals, weighed, and sieved to determine grain size distribution. Samples of the bed surface were recovered at the end of each run by removing plates (0.5 m long, 0.3 m wide) which had previously been buried in the flume bed. The undisturbed bed surfaces on these plates were fixed with glue and analysed for grain size distribution by measuring grains located at fixed points on an overlaid grid²⁰. Water surface and bed slope were determined every 6 min by reading point gauges placed at the 1-m intervals along the flume centreline. The mean water surface slope of each run decreased with reduced sediment loading, from 0.0052 to 0.0046 and then to 0.0035 (all $\pm 15\%$), while the mean depth increased from 10 to 11 cm. Runs lasted six to eight hours, and were terminated at equilibrium when water surface slope stabilized and when rate and size distribution of bedload discharge matched those of bedload supply.

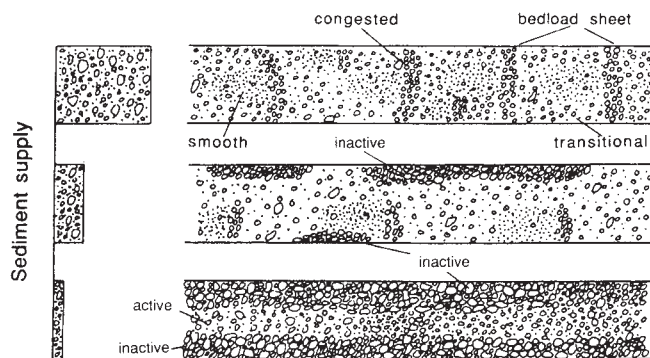


FIG. 2 Plan view of the flume bed, showing effect of sediment loading rate (indicated by 'supply' box) on lateral and longitudinal grain size segregation of flume bed surface (flow is left to right). At a high (17.4 g min^{-1} per cm width) sediment supply rate, bedload travelled as thin rapidly migrating bedload sheets (see text) with relatively well sorted coarse (4.7 mm median diameter) 'congested' leading edges, followed by poorly sorted 'smooth' and 'transitional' zones (2.7 mm and 3.7 mm median diameter, respectively). Reductions in sediment supply resulted in expansion of the coarse 'inactive' zones, in which little or no transport took place.

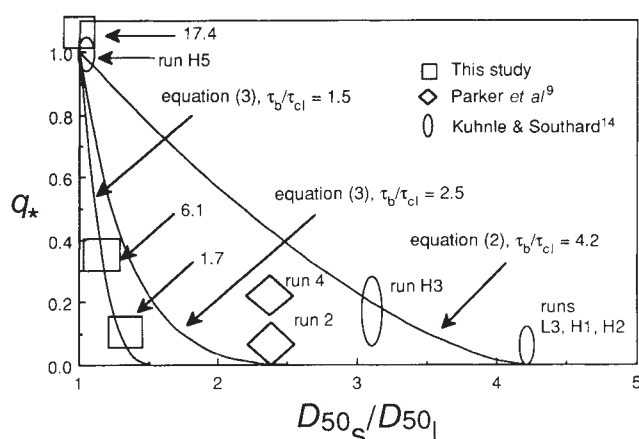


FIG. 3 Comparison of the predicted transport ratio q_* as a function of median grain diameter (D_{50}) ratio, with the observed transport ratio and grain ratio at transport rates of 17.4, 6.1, and 1.7 g min^{-1} per cm width. The symbol size represents the approximate range of observed values for each run.

and widening of high- q_* zones. In streams with high initial q_* values, grain size adjustment resulting from increased load is limited, and hence increased bedload supply should lead to net deposition and consequently to altered channel morphology, such as pool infilling and channel migration. If accelerated erosion leads primarily to increased fine bedload supply, it should cause fining of the bed, which may in turn lead to mobilization of the coarser fraction and an increase in q_* . These hypotheses are being tested in continuing field studies on California rivers.

In most rivers, many factors (limited supply of gravel-size material to streams from hillslopes, chemical and mechanical breakdown of bed material, net loss of material to floodplains, and increasing distance downstream from upland sediment sources) restrict bedload supply and promote bed surface coarsening. On a very local scale, topographically induced flow divergence and cross-channel rolling of particles can reduce the sediment supply and coarsen individual patches of the bed surface^{18,19}. Our sediment-supply hypothesis suggests a continuum of surface responses, from the well-armoured river beds below dams (where all bedload supply is eliminated) to the armour-free surface found on bars below landslides or other local high-supply sources. □

A new, post-stishovite high-pressure polymorph of silica

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THE possibility of 'post-stishovite' phases of silica (SiO_2) has been the subject of extensive study during the past two decades, not only because of the intrinsic crystallographic interest but also because of its relevance to the geochemistry of the lower mantle. Here *in situ* X-ray diffraction observations of SiO_2 compressed to pressures of up to 124 GPa using a laser-heated diamond-anvil cell. Stishovite was formed by heating α -quartz or amorphous silica at pressures of up to 92 GPa; on heating at 108 and 124 GPa, however, the materials crystallized into a CaCl_2 -type structure, which results from a distortion of the stishovite structure and is slightly denser. The quenched and recovered sample was always stishovite; thus the transformation from stishovite to the CaCl_2 structure is reversible on release of pressure.

Silica is one of the most abundant minerals in the Earth's crust and occurs in many different forms, or polymorphs. Since the discovery of the rutile-type polymorph (stishovite) above 10 GPa, many attempts have been made to find further high-pressure polymorphs of SiO_2 . Both α - PbO_2 -type and Fe_2N -type SiO_2 have been synthesized at very high pressures using either shock or static compression^{1,2}, but neither polymorph has yet been confirmed to exist as a stable phase. Based on systematic studies of AX_2 -type compounds, it is generally believed that stishovite may ultimately transform into an eightfold-coordinated fluorite structure³.

A recent theoretical prediction using a first-principles electronic structure calculation⁴ has indicated, however, that fluorite-type SiO_2 has a much higher free energy than stishovite, making it an unlikely candidate for the 'post-stishovite phase'. Instead, it was pointed out that stishovite may ultimately transform into a $\text{Pa}\bar{3}$ (pyrite-like) structure; the aim of our experiments was to test this theoretical prediction.

Synthetic single crystals of quartz, natural quartz sand and amorphous silica were used as starting materials. These samples were finely ground and mixed with platinum black, which absorbs the Nd YAG laser light. Details of the experiments are described elsewhere⁵. Powdered samples were embedded in a small hole (100 μm) in a stainless-steel gasket and concentric

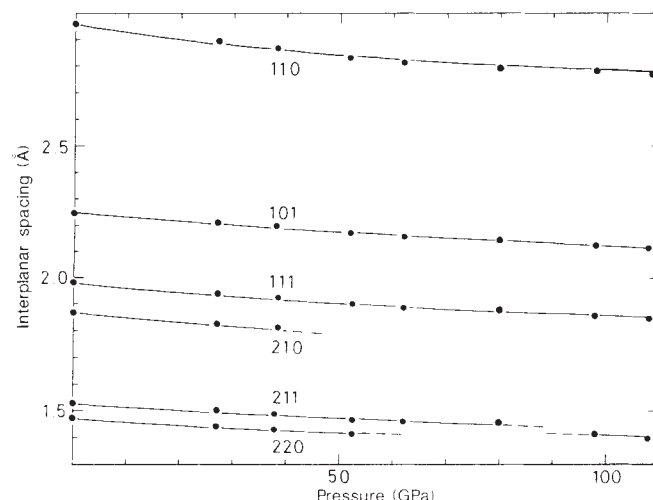


FIG. 1 Interplanar spacing of SiO_2 observed after α -quartz and amorphous silica have been heated at each pressure.

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TABLE 1 X-ray diffraction data

Run no.	Pressure (GPa)		X-ray data		Lattice parameters (Å), (Å ³)	Calculated unit cell	
	Before heating	After heating	<i>h k l</i>	<i>d</i> -spacing (Å)		<i>c/a</i>	<i>V/V</i> ₀
			Rutile structure				
880330	29.6 (26–33)*	26.4 (25–28)	1 1 0	2.892 (2)	<i>a</i> =4.091 (1)†	0.642 (2)	0.944 (1)
			1 0 1	2.207 (2)	<i>c</i> =2.626 (2)		
			1 1 1	1.945 (1)	<i>V</i> =43.94 (5)		
			2 1 0	1.831 (2)			
			2 1 1	1.502 (1)			
			2 2 0	1.446 (2)			
880620	45.3 (43–48)	37.7 (36–38)	1 1 0	2.863 (5)	<i>a</i> =4.060 (2)	0.644 (2)	0.926 (2)
			1 0 1	2.199 (3)	<i>c</i> =2.613 (4)		
			1 1 1	1.932 (2)	<i>V</i> =43.08 (8)		
			2 1 0	1.819 (4)			
			2 1 1	1.491 (2)			
			2 2 0	1.435 (3)			
880426	64.3 (61–69)	52.2 (49–58)	1 1 0	2.834 (5)	<i>a</i> =4.016 (6)	0.644 (3)	0.904 (4)
			1 0 1	2.176 (4)	<i>c</i> =2.586 (6)		
			1 1 1	1.910 (4)	<i>V</i> =41.72 (16)		
			2 1 1	1.476 (2)			
880709	72.8 (68–80)	62.0 (56–70)	1 1 0	2.826 (5)	<i>a</i> =4.002 (14)	0.640 (5)	0.874 (8)
			1 0 1	2.163 (4)	<i>c</i> =2.561 (12)		
			1 1 1	1.893 (5)	<i>V</i> =41.02 (35)		
			2 1 1	1.486 (2)			
880626	92.1 (88–98)	79.5 (75–85)	1 1 0	2.792 (6)	<i>a</i> =3.960 (14)	0.646 (5)	0.861 (8)
			1 0 1	2.153 (6)	<i>c</i> =2.557 (13)		
			1 1 1	1.882 (5)	<i>V</i> =40.09 (36)		
			2 1 1	1.457 (2)			
			CaCl ₂ structure				
880510	108 (105–113)	98.2 (97–100)	1 1 0	2.784 (6)	<i>a</i> =4.097 (5)		0.832 (1)
			1 0 1	2.127 (6)	<i>b</i> =3.795 (5)		
			1 1 1	1.856 (4)	<i>c</i> =2.559 (1)		
			1 2 1	1.416 (3)	<i>V</i> =38.07 (5)		
880719	124 (115–130)	108 (100–115)	1 1 0	2.766 (7)	<i>a</i> =4.109 (36)		0.815 (8)
			1 0 1	2.113 (6)	<i>b</i> =3.741 (9)		
			1 1 1	1.844 (4)	<i>c</i> =2.468 (9)		
			1 2 1	1.401 (3)	<i>V</i> =37.93 (37)		

* The range of pressure in the irradiated region is shown in parentheses.

† The values in parentheses are standard deviations.

distributions of pressure were observed. Average pressures in the region irradiated by X-rays (70 μm across) were measured using ruby fluorescence, calibrated up to 170 GPa (ref. 6). Three to five small ruby chips were placed on top of the samples, to avoid reaction with SiO₂. The radial pressure distribution was derived by fitting a smooth curve to these observations, and average pressures weighted by areas were used for the analysis. Samples were heated to >1,000 °C using the laser; the sample temperature was estimated from the light emitted by the sample.

For each sample three X-ray measurements were made: first at the desired pressure and room temperature; second, after the sample had been heated and quenched at high pressure; and, third, at ambient conditions, after the pressure was released. High-pressure *in situ* X-ray observations were made using a high-power, rotating-anode X-ray source operated at 55 kV and 160 mA. Filtered Mo Kα radiation was collimated by a 60-μm pinhole and the diffracted X-rays were measured by a Debye-Scherrer camera with a radius of 57.3 mm. The typical exposure time was three days. The recovered samples were examined using Co Kα radiation and a Debye camera with a 66 mm radius, to obtain higher resolution and accuracy.

The experimental results are summarized in Table 1. Figure 1 shows the interplanar spacings (*d*) of the quenched samples at high pressure. Below 80 GPa, all of the *d*-values are characteristic of the rutile structure. The choice of starting material (quartz or amorphous silica) had no effect on the results. The

absence of large changes in the diffraction pattern shows that transformations into the α-PbO₂-type, Fe₂N-type or *Pa* $\bar{3}$ -type polymorphs do not occur under these conditions. For the materials synthesized at 108 and 124 GPa (98 and 108 GPa after quenching), however, there are significant (3%) discrepancies between the observed and calculated *d*-values. Below these pressures, the discrepancies were less than 0.3% (Table 2). At very high pressures, because of the pressure gradients and non-hydrostatic stresses in the sample, the lines became broader and the accuracy of the measured *d*-values is diminished, but the observed discrepancies are much larger than the uncertainties caused by the broadening of the lines. In Figs 2 and 3, the volume and lattice parameters of the tetragonal unit cell (rutile structure) are plotted as solid symbols. The data define a smooth curve up to 80 GPa, suggesting that stishovite is stable up to this pressure.

The dashed line in Fig. 2 shows the pressure-volume relationship for stishovite (bulk modulus, *K*₀=298 GPa and (*dK/dP*)₀=0.7) obtained by an X-ray diffraction study using a cubic-anvil high-pressure apparatus under hydrostatic pressures up to 11 GPa (ref. 7). A similar bulk modulus, *K*₀=306 GPa, has been derived from a Brillouin scattering measurement⁸. Our compression data give a bulk modulus that is larger than these two values but which is in reasonable agreement with that obtained using a diamond-anvil cell⁹ (the dot-dashed line in Fig. 2). In experiments using the diamond-anvil cell, pow-

TABLE 2 Observed and calculated d -values for the two structures at 98 and 108 GPa

Rutile structure				CaCl ₂ structure			
$h\ k\ l$	d_{obs}	d_{cal}	$d_{\text{obs}}/d_{\text{cal}} - 1$	$h\ k\ l$	d_{obs}	d_{cal}	$d_{\text{obs}}/d_{\text{cal}} - 1$
$P=98\text{ GPa}$							
1 1 0	2.784	2.711	0.027	1 1 0	2.784	2.784	0.000
1 0 1	2.127	2.115	0.006	1 0 1	2.127	2.127	0.000
1 1 1	1.856	1.852	0.002	1 1 1	1.856	1.856	0.000
2 1 1	1.416	1.420	-0.004	1 2 1	1.416	1.416	0.000
$P=108\text{ GPa}$							
1 1 0	2.766	2.682	0.031	1 1 0	2.766	2.766	0.000
1 0 1	2.113	2.100	0.006	1 0 1	2.113	2.115	-0.001
1 1 1	1.844	1.837	0.004	1 1 1	1.844	1.842	0.001
2 1 1	1.401	1.408	-0.005	1 2 1	1.401	1.401	0.000

dered samples are directly compressed between two flat diamond surfaces, so that large pressure gradient is present, as well as a uniaxial stress. Under these circumstances, ruby fluorescence is sensitive to the highest stress state whereas the d -values vary in a complicated manner depending on the angle between the compression axis and the crystallographic axis of anisotropic crystals; there may therefore be differences between the results of hydrostatic and diamond-anvil-cell experiments. To get a reliable equation of state for such an anisotropic material, it may be necessary to use solidified gas as a quasi-hydrostatic pressure-transmitting medium¹⁰.

Figures 1 and 2 suggest that a distortion of stishovite occurs between 79 and 98 GPa. Among the many possible distortions of the rutile structure, we found that the orthorhombic CaCl₂ structure is consistent with the experimental results (Table 2). A lattice-dynamics calculation¹¹ and a molecular-dynamics study¹² also suggest that stishovite undergoes a pressure-induced second-order phase transition to the CaCl₂ structure. Furthermore, such a transition at ~ 100 GPa was suggested by high-pressure Raman spectroscopic measurements¹³, which showed that stishovite has a pressure-induced soft mode that connects the two structures. The results of unit-cell calculations based on this structure are summarized in Table 2, and Figs 2 and 3; the observed d -values are consistent with the CaCl₂ structure. To explain the intensities, however, it is necessary to take into account the effect of preferred orientation. When a solid sample is compressed in a diamond-anvil cell, a large uniaxial stress component exists and results in a preferred orientation of the

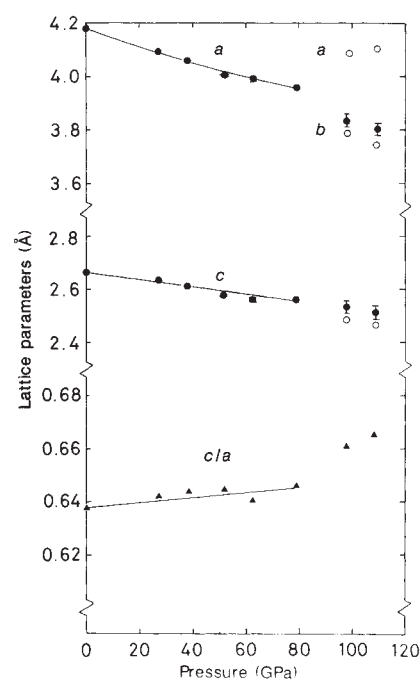


FIG. 3 Pressure dependence of lattice parameters and axial ratio of SiO₂. Solid and open symbols as in Fig. 2.

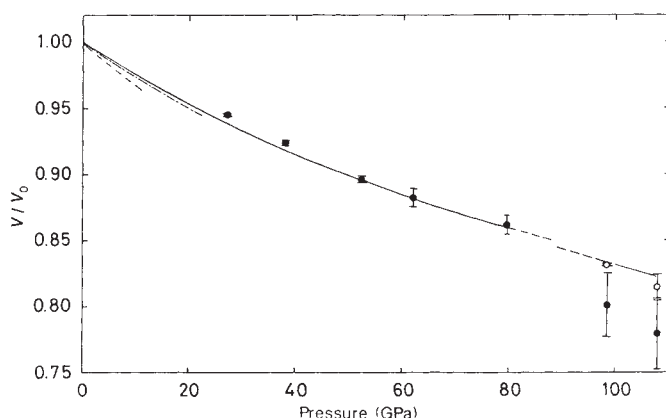


FIG. 2 Equation of state of SiO₂. Solid symbols are the results obtained by assuming the rutile structure (stishovite); open symbols are derived using the CaCl₂-type structure. The dashed line shows the pressure-volume relationship for stishovite ($K_0=298\text{ GPa}$ and $(dK/dP)_0=0.7$) obtained in a previous X-ray diffraction study using a cubic tungsten-carbide-anvil cell under hydrostatic pressure up to 11 GPa (ref. 7). The dot-dashed line is the result obtained using a lever-and-spring diamond-anvil cell⁹.

powdered sample. In the present case, the observed diffraction pattern can be explained by assuming that the a axes of the grains are preferentially aligned parallel to the compression axis. At ambient conditions all the recovered samples have the same unit cell as the rutile structure and no differences were found among the samples recovered from below and above 80 GPa. This fact suggests that the transformation into the CaCl₂ structure is reversible and therefore unquenchable.

We conclude that these results can be explained by the reversible distortion of the rutile structure into the CaCl₂ structure, and the preferred orientation of grains under a large uniaxial stress. Only four diffraction lines, however, were observed above 100 GPa, and so the possibility of another type of distortion cannot be ruled out completely. It is also unclear whether or not this transition would occur under purely hydrostatic conditions. The density change associated with this transformation is very small and $\Delta V/V_0$ is estimated to be less than 1%.

Knittle and Jeanloz have shown that (Mg, Fe)SiO₃ perovskite is stable under the conditions of the lower mantle¹⁴. At present, it can be concluded that, under the conditions in the Earth's lower mantle, SiO₂ crystallizes as stishovite or a very similar phase. If stishovite were to transform into a much denser structure, the assemblage of SiO₂ plus MgO might become denser

than MgSiO_3 perovskite. Our results demonstrate, however, that perovskite is denser than the isochemical mixture of oxides throughout this pressure range and support the suggestion that the MgSiO_3 perovskite is the dominant mineral phase throughout the lower mantle. $\square$

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Organic materials in a martian meteorite

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THE meteorite EETA 79001, which many believe to have originated on Mars, contains carbonate minerals thought to be martian weathering or alteration products. Accompanying the carbonates are unexpectedly high concentrations of organic materials (defined here as carbonaceous matter that has a low stability towards oxidation, and so combusts at $<600^\circ\text{C}$; the term 'organic' does not necessarily imply an origin by biogenic processes). Although the carbon isotope composition of these materials is indistinguishable from terrestrial biogenic components, and so cannot be used to assess the source, we argue here that their occurrence in an interior sample of a clean Antarctic meteorite militates against a wholly terrestrial origin. A sample of martian organic materials may thus be available for further study in the laboratory.

The Viking molecular-analysis experiment showed that within detection limits (that is, better than 1 part per 10^9 (p.p.b.)) the surface soils of Mars do not contain organic molecules¹. This observation allows the apparently positive results from biological investigations on the Lander² to be understood without requiring the existence of metabolizing organisms at the surface of the planet³. The dearth of organic materials in martian soil is probably a result of their destruction by various photochemical processes^{4,5}. These are purely surface phenomena, however, and so it is still possible that organic compounds, either abiogenic materials or the fossil residues of biological activity, could be present at depth within the martian crust. Samples of martian crustal material are believed to be present on Earth in the form of SNC meteorites (the salient arguments in favour of a martian origin for this achondritic group of meteorites are dealt with in refs 6 and 7); the nature of the carbon present on Mars may thus be determined through detailed laboratory studies of these materials. Superficially, it might be expected that these mafic igneous rocks (see ref. 7 for petrological details) could give information concerning only magmatic processes on Mars. In addition to containing samples of trapped martian atmosphere^{8,9}, presumably shock-implanted during ejection of the meteorites, it is apparent, however, that SNC meteorites also

contain preterrestrial alteration products, such as iddingsite¹⁰, and martian weathering products such as volatile-rich aluminosilicates¹¹ and carbonates^{12–15}. Closer examination of data obtained previously from the EETA 79001 shergottite¹⁵ suggests that the volatile-rich fractions of this sample also contain a large amount of carbonaceous materials which are interpreted as organic in nature.

During a survey of carbon in SNC meteorites, a subsample of EETA 79001 from lithology B, referred to henceforth as lith. B) was found to have an extremely high carbon content (740 p.p.m.), almost as much as the two nakhlites, Governorador Valadares and Lafayette¹², which are known to have suffered weathering and contamination on Earth. As EETA 79001 was collected under extremely clean conditions in the Antarctic¹⁶, and as two further subsamples (from lithologies A and C) contained the lowest carbon contents (124 and 48 p.p.m. respectively) of all SNC meteorites, the enrichment of carbon in lith. B was very unusual. During analysis by stepped combustion¹⁷, ~80% of the total carbon in lith. B was liberated below 600°C , that is, over the temperature regime where organic materials combust^{17,18}. The conclusion¹² that the high abundance of carbon in lith. B was the result of laboratory contamination may have been premature as other fractions of EETA 79001 have been found to contain large amounts of indigenous low-temperature carbon. Indeed, results from E,239, a subsample of EETA 79001 from a lith. A/C contact deep within the meteorite (analysed initially because of its visual coating of carbonate minerals¹³), strongly suggest the presence of large amounts of preterrestrial organic materials.

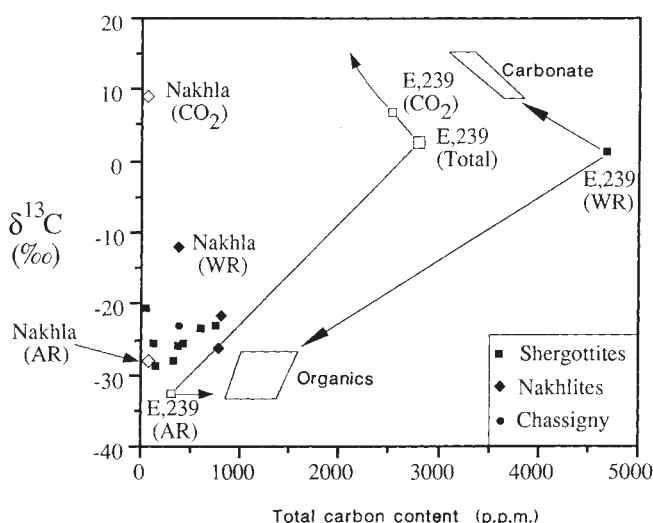


FIG. 1 Plot of $\delta^{13}\text{C}$ against carbon content of SNC meteorites. The filled symbols represent data from whole-rock (WR) samples. The open symbols represent the data from meteorite fractions, that is, either carbon dioxide released by acid treatment (CO_2), thought to be representative of the carbonate minerals, or the acid residue (AR) remaining from this treatment. In the case of E,239 the complementary data from acid extraction and analysis of the acid residue are summed (Total). The discrepancy between the location of this point and the whole-rock datum is discussed in the text. Note that the CO_2 liberated by acid treatment is somewhat lighter than the most extreme values measured by stepped combustion of the same sample (see Fig. 2). Assuming that E,239 (Total) is a two-component mixture of AR-like (organic) carbon and carbonate carbon, then as $\delta^{13}\text{C}$ of the CO_2 approaches a value of $+15\%$ (the maximum measured thus far for carbonate minerals in SNC meteorites) the relative quantity of organic carbon increases to ~700 p.p.m. (arrows emanating from the AR and CO_2 points). Using reasonable estimates of the isotopic composition of organic and carbonate carbon, E,239 (WR) can be considered to represent a two-component mixture, corresponding to the two boxes (labelled Carbonate and Organics). On the basis of this calculation, E,239 could contain 870–1,540 p.p.m. organic carbon, which is well in excess of the carbon content of any other whole-rock SNC meteorite.

Figure 1 shows a plot of $\delta^{13}\text{C}$ against total carbon content for all SNC meteorites^{12,15,19}. The whole-rock data (filled symbols) generally occupy the region between $\delta^{13}\text{C} = -30$ and -20% , with carbon contents of <750 p.p.m. However, two samples, Nakhla and E,239, are characterized by elevated whole-rock $\delta^{13}\text{C}$ values^{12,15}, caused by the presence of carbonate minerals which have $\delta^{13}\text{C}$ of up to $+15\%$. The E,239 whole-sample datum plots well away from the other SNC meteorites at $\delta^{13}\text{C} = +1.2\%$ and 4,680 p.p.m. carbon.

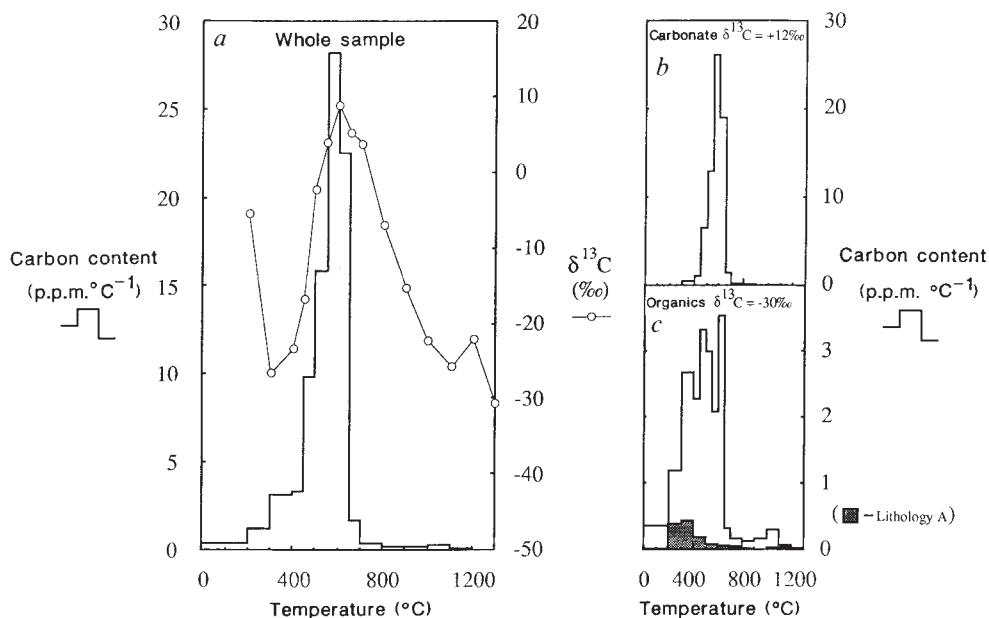
The open symbols in Fig. 1 represent either data acquired as CO_2 liberated from the meteorite fractions following treatment with orthophosphoric acid (denoted CO_2 in Fig. 1), or analyses of the acid-resistant residues remaining after this extraction (AR in Fig. 1). Orthophosphoric acid is used to selectively attack carbonate minerals, liberating CO_2 gas; $\delta^{13}\text{C}$ of the carbonate lies between $+7\%$ (E,239) and $+9\%$ (Nakhla). The carbon remaining after acid treatment has a combustion temperature that is consistent with its identification as organic material with $\delta^{13}\text{C}$ of -28% (Nakhla) or -33% (E,239). When CO_2 and acid-resistant residue data acquired from E,239 are summed, a material balance is not obtained; the total (Fig. 1) is $\sim 2,200$ p.p.m. less than that measured from the whole sample. This can be interpreted in a number of ways: (1) the forms of carbon are heterogeneously distributed; (2) some carbon was lost while reclaiming the acid-residue; (3) a portion of the carbon remained in solution in the acid; or (4) loss of volatile forms of carbon (as gases other than CO_2) during acid dissolution. In practice, a combination of these effects is probable, although the first point (which is addressed below) is likely to be the most significant. There is an additional complication here because the $\delta^{13}\text{C}$ of the CO_2 liberated by the acid from E,239 is somewhat less than values measured during stepped combustion ($+9\%$; see Fig. 2). This suggests that the CO_2 liberated from the whole sample by acid treatment is contaminated by isotopically light CO_2 released concurrently from the organic fraction. A similar effect is observed in the Nakhla data, where the maximum $\delta^{13}\text{C}$ obtained during stepped heating was $+15\%$ (ref. 12). Assuming that E,239 (total) is a two-component mixture of carbonate and organics, then as $\delta^{13}\text{C}$ of the carbonate approaches $+15\%$ the concentration of organics (assuming a $\delta^{13}\text{C}$ of -33%) increases from 320 to more than 700 p.p.m. (represented in Fig. 1 as the arrows moving away from the points labelled CO_2 and AR, the latter denoting the sum of acid-soluble/degradable and acid-insoluble organic carbon). In other

words more than 400 p.p.m. of carbon in E,239 could be acid-soluble/degradable organic material.

To constrain the carbon distribution in E,239 further, it is appropriate to consider the stepped-combustion data (Fig. 2). From 450°C to 700°C there is a major release of ^{13}C -rich carbon ascribed to decrepitation of carbonate minerals, but the $\delta^{13}\text{C}$ values do not form a plateau, demonstrating that additional components liberate CO_2 over the same temperature range. From 200°C to 450°C there is a partially resolved release of carbon (considerably smaller than that from the carbonate) with $\delta^{13}\text{C} \leq -27\%$; this is from the combustion of organic materials. As the 200 – 700°C release constitutes $\sim 96\%$ of the total carbon, the whole sample may be considered a two-component mixture. Assuming reasonable estimates of the end-member isotopic compositions ($\delta^{13}\text{C} = +9$ to $+15\%$ for carbonate, that is, the maxima obtained during stepped heating of E,239 and Nakhla, and $\delta^{13}\text{C} = -33$ to -27% for the organics) the concentrations of these components plot in the boxes marked in Fig. 1. The total content of carbon as organic materials in E,239 is thus 870–1,540 p.p.m. This is higher than any other whole-rock SNC meteorite and considerably in excess of that expected from normal laboratory or handling contamination (the maximum levels of an extraction-system blank would be equivalent to ~ 80 p.p.m.). The organic-carbon content calculated here and the maximum calculated previously (~ 700 p.p.m.) represents a fairly close agreement; the slight discrepancy is probably a reflection of sample heterogeneity, although carbon losses during the acid treatment cannot be ruled out. The difference between the material balance and the whole-sample data (Total and WR in Fig. 1) can thus be reasonably understood in terms of heterogeneous distribution of carbonate minerals, with organic compounds present in approximately equivalent concentrations. In Fig. 2 the whole-sample data for E,239 is deconvoluted into calculated releases for carbonate and organics ($\delta^{13}\text{C} = +12\%$ and -30% respectively, both values selected to be the medians of the anticipated ranges). As the bulk of the E,239 sample is lith. A, which does not contain any significant quantities of carbonate minerals¹⁹, the calculated organic release for E,239 may be compared with data acquired by stepped combustion of lith. A. The concentration of organic materials in E,239 is an order of magnitude greater than that in lith. A (Fig. 2) (indeed, the profile for lith. A can be considered as an upper limit for terrestrial organic contamination).

The E,239 sample was excavated from deep within the

FIG. 2 *a*, Stepped-combustion data for E,239. A single major release of carbon is apparent between 300°C and 700°C . That this is not from a single component can be discerned by the change in $\delta^{13}\text{C}$ across the release, from a value as low as -27% to one as high as $+9\%$. Assuming that the sample is a two-component mixture of carbonate ($\delta^{13}\text{C} = +12\%$) (*b*) and organics ($\delta^{13}\text{C} = -30\%$) (*c*) allows the release due to each component to be calculated. Note that the scale for the carbonate release is ~ 10 times greater than that due to the organics (the sample was analysed originally because of its high carbonate content). For comparison, the carbon released from lith. A is superimposed on the release from organics and shows the enormous enrichment of low-temperature carbon in the E,239 sample.



meteorite and, given its obviously unusual nature, the material has been handled very carefully; it should therefore contain only a minimal amount of terrestrial organic contamination. The carbonate minerals in E,239 are thought to be preterrestrial (and, by inference, martian) in origin¹³⁻¹⁵, and in view of the careful handling we must conclude that the accompanying organic materials must also have a similar origin. This might therefore constitute the first recognition of organic compounds from Mars, which proved so elusive during the remote analyses by Viking. We advocate that samples like E,239 be subjected to further isotopic investigations (for example, D/H) and organic-analytical techniques, in order to discern exactly which compounds are present and to ascertain their origins. The implications for studies of Mars are obvious. □

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Neodymium and lead isotope evidence for enriched early Archaean crust in North America

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THERE has been considerable debate as to whether isotopically enriched continental crust was an important geochemical reservoir in earliest Archaean times. Fundamental questions such as the volume of the early crust, its composition and the effect of its formation and/or recycling on mantle geochemistry, have still to be answered. The most direct clues to the nature of the earliest crust and mantle are obtained from the isotope systematics of old rocks¹⁻⁵. Here we report neodymium and lead isotope measurements and uranium-lead zircon geochronology from Archaean gneisses of the Slave Province, Northwest Territories, Canada. The gneisses contain zircons with cores older than 3.842 Gyr. One sample of tonalitic gneiss has a Nd chondritic model age of 4.1 Gyr and an ϵ_{Nd} (3.7 Gyr) of -4.8. This is the oldest reported chondritic model age for a terrestrial sample and provides evidence for strongly enriched pre-3.8-Gyr crust, a reservoir complementary to the depleted mantle already in existence by 3.8 Gyr before present^{2,6}.

The Slave Province is an Archaean granite supracrustal terrane in the north-western part of the Canadian Shield (Fig. 1). The supracrustal rocks, collectively termed the Yellowknife Supergroup (Sgp)^{7,8}, are predominantly metaturbidites but also include mafic to felsic metavolcanic rocks. U-Pb zircon ages from the Slave Province suggest that most of the metavolcanic rocks were deposited between 2.65 and 2.70 Gyr and were intruded by 2.58 to 2.67 Gyr granitic to dioritic plutons⁹⁻¹¹. Granites and gneisses predating the Yellowknife Supergroup, as well as numerous remnants of supracrustal rocks (whose relationship with the older units is uncertain) occur only in the western part of the Slave Province (Fig. 1). Much of these older units consists of heterogeneous tonalitic to granitic gneisses¹²⁻¹⁴, with ages of 2.98-3.15 Gyr (refs 13, 15). Furthermore, Dudas *et al.*¹⁶ have reported Nd isotope data suggesting the presence of crust as old as 3.3 Gyr.

The rocks sampled for this study occur in the westernmost Slave Province where they are exposed within the foreland and metamorphic hinterland of the early Proterozoic Wopmay Orogen¹⁷⁻¹⁹ in a set of Proterozoic structural culminations (Fig. 1). Pink, massive to foliated granite with rare, metre-scale xenoliths of amphibolitic to granitic gneisses underlie the eastern culmination. The number and size of gneissic xenoliths increase westward across the central culmination and on the west side of the culmination the Archaean rocks are predominantly massive gneissic units which extend across the western culmination. The samples taken for isotope analysis and dating in the present study are from the massive gneisses in the structural saddle between the central and western culminations (Fig. 1).

Four non-magnetic fractions of clear, euhedral zircons were separated from a sample of tonalitic gneiss (BG-83-A) and analysed for their U and Pb isotopic composition (Table 1a). These analyses yield an upper intercept age of 3.480 ± 0.008 Gyr (Fig. 2a). Additional, more magnetic zircons from this sample were analysed, including abraded grains, and it is apparent from

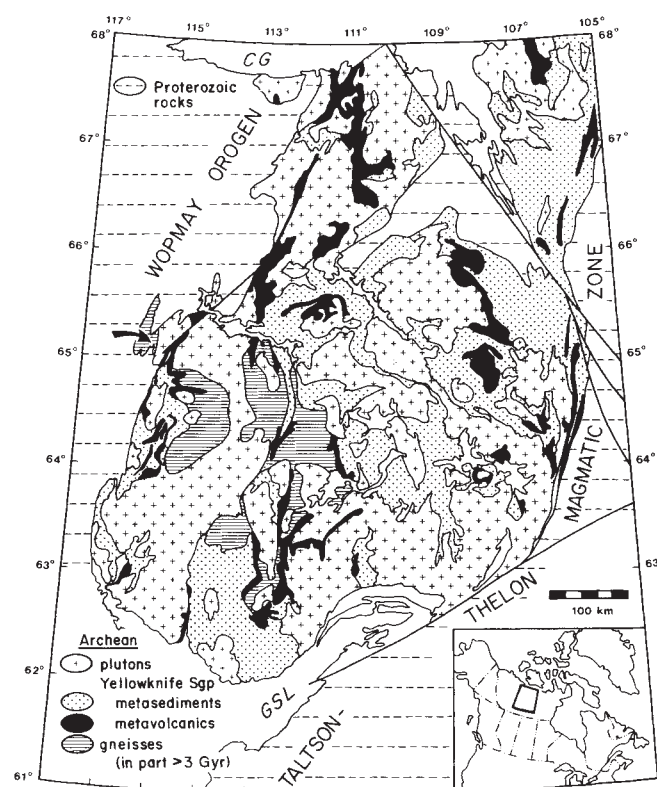


FIG. 1 Generalized geological map of the Slave Province, after Hoffman²⁹. Localities of samples analysed in this study are shown by the arrow.

the scatter introduced by the abraded points that there is probably an older xenocrystic component present. The upper intercept age of the four non-magnetic fractions must be viewed as a maximum age of the young component and the abraded sample with the oldest Pb-Pb age (3.496 Gyr) provides a minimum estimate of the age of the inherited component.

A sample of layered amphibolitic-tonalitic gneiss (BGXM), about 10 m away from BG-83-A, yielded a very heterogeneous population of zircons which range from large (10–250 μg) dark grains and fragments to fine clear grains. Many of the large grains have visible metamict cores as well as abundant opaque inclusions. Eleven fractions of the large zircons, ranging from one to five grains, were analysed. An attempt was made to isolate the core material by abrasion and mechanical breakage of the grains. The isotope data for the zircons are presented in Table 1 and are scattered in U-Pb space (Fig. 2b). Fraction 12 has a Pb-Pb age of 3.842 Gyr and must be considered the minimum age of the oldest component. The scatter is considered to result from mixing of older (core) and younger (rim) component(s) and/or complex Pb-loss history. Fraction 8 has a Pb-Pb age of 3.793 Gyr but plots to the right of the main array of data points, suggesting that it contains a component of discordant but much older material. The age of the youngest component is difficult to determine exactly but is older than 3.651 Gyr, the Pb-Pb age of fraction 11 (which is comprised of clear zircons having no visible cores). The simplest interpretation of the U-Pb zircon data from both samples is that magmatism around 3.5–3.7 Gyr ago involved an older protolith, incorporating xenocrystic zircons of age in excess of 3.842 Gyr.

BGXM is a foliated orthogneiss consisting of tonalitic and amphibolitic layers alternating on a centimetre scale. The tonalitic layers consist of granoblastic plagioclase and quartz, with relict porphyroclasts of plagioclase (anorthite content of

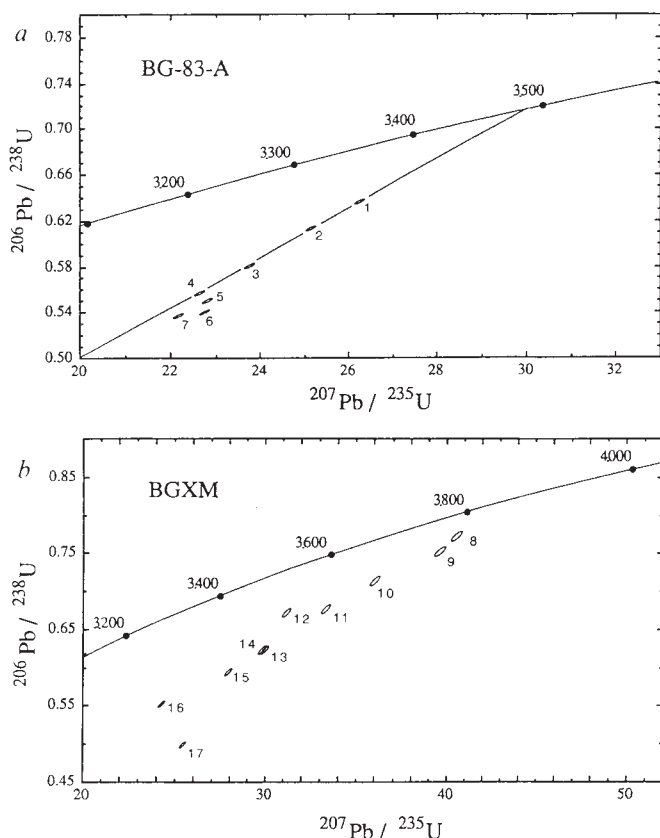


FIG. 2 a, Concordia diagram for zircons from gneiss sample BG 83 A. Ages, in Gyr, are marked on upper curve. b, Concordia diagram for zircons from gneiss sample BGXM. Ages, in Gyr, are marked on upper curve.

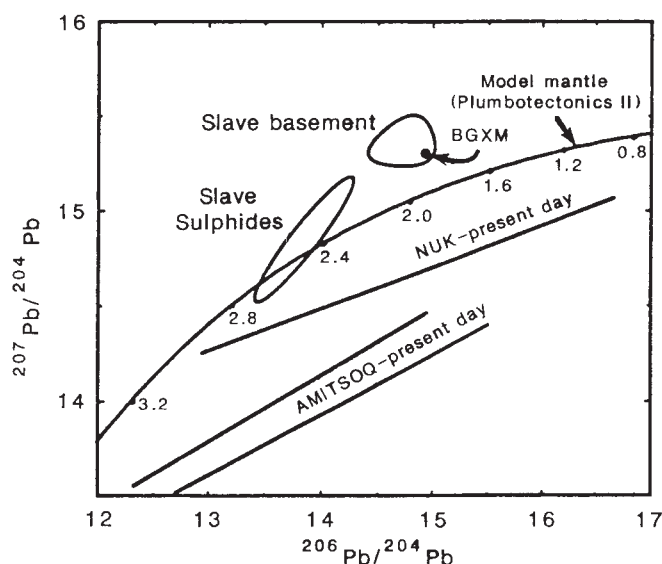


FIG. 3 Summary of the $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ relationships between remnants of old (>3.0 Gyr) gneisses and ~2.7 Gyr massive sulphides from Slave Craton; data from the Nuk and Amitsoq gneisses, Greenland are shown for reference. The Slave gneiss field is defined by the BGXM analysis reported here and unpublished feldspar data from a variety of 3.0–3.5 Gyr gneisses in the western Slave Province. Slave sulphide data are from syngenetic massive sulphide deposits within ~2.7 Gyr greenstone belts (compiled by Franklin and Thorpe²⁰). Data from the Greenland Archaean gneisses are whole-rock measurements^{21–23}. The model mantle curve is from Plumbotectonics II (Zartman and Doe³⁰).

approximately An_{30}) and minor hornblende and biotite. The amphibolitic layers consist of aligned hornblende and biotite, plagioclase, quartz and sphene with accessory allanite. Zircon occurs in both layers. A 0.5-kg slab of gneiss was sawed into two pieces, a banded portion (BGXM) and an amphibolitic portion (BGXM-M). An aliquot of BGXM whole-rock powder (Table 1b) yields an 'initial' ϵ_{Nd} (3.7 Gyr) of -4.8. (see Table 1 legend). We believe an age of 3.7 Gyr is a reasonable estimate of the age of the younger component of the zircons in this rock; although it must be viewed with caution, ϵ_{Nd} as a function of time would still be distinctly negative (-3) at 3.9 Gyr. The value at 3.7 Gyr (ϵ_{Nd} (3.7) = -4.8) indicates that the protoliths of the gneisses had a time-integrated $^{147}\text{Sm}/^{144}\text{Nd}$ ratio considerably lower than chondritic. A chondritic model age for this sample is 4.1 Gyr, which is the oldest known terrestrial model age. This model age is consistent with the zircon data, which require the involvement of >3.844-Gyr-old crust. An aliquot of BGXM-M yields an ϵ_{Nd} (3.7) of -2.6, and a chondritic model age of 3.9 Gyr. This material is slightly different in its isotopic composition, which we interpret as reflecting original heterogeneity in the gneiss protolith, not metamorphic differentiation.

The isotopic composition of the least radiogenic Pb in plagioclase feldspars from this rock (Table 1a) and other basement rocks in Slave Craton are extremely elevated relative to the mantle growth curve (Fig. 3), which suggests evolution in an environment with relatively high μ ($^{238}\text{U}/^{204}\text{Pb}$) before formation of the gneisses at 3.5–3.7 Gyr. The elevated initial feldspar Pb values are consistent with involvement of very old crust and support the zircon and Nd data. The location of the field of basement gneisses on Fig. 3 supports the interpretation of Franklin and Thorpe²⁰ that the array of Pb isotope values from the Slave Province massive sulphides represents mixtures of mantle-derived Pb and old basement Pb. Also shown in Fig. 3 is the field of data from the Archaean crust of Greenland, which in contrast has an extremely depleted Pb isotopic signature^{21–23} and no evidence for crust much older than 3.8 Gyr (ref. 2).

TABLE 1 Isotope data

Zircon fractions*			Concentrations		(a) U-Pb					Age (Gyr)
					Measured	Atomic ratios corrected for blank and common Pb				
No.	Properties	Weight (mg)	U (p.p.m.)	Pb (p.p.m.)	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁶ Pb	²⁰⁶ Pb/ ²³⁸ U	²⁰⁷ Pb/ ²³⁵ U	²⁰⁷ Pb/ ²⁰⁶ Pb	²⁰⁷ Pb/ ²⁰⁶ Pb
BG-83-A										
1	nm1†	1.9	433	330	3,690	0.08044	0.63638	26.25740	0.29925	3.466
2	m-1†	1.7	536	393	3,300	0.07726	0.61339	25.14380	0.29730	3.456
3	m0†	1.6	657	456	3,610	0.07849	0.58057	23.74970	0.29669	3.453
4	m1†	2.5	658	439	3,448	0.08064	0.55673	22.64700	0.29503	3.444
5	m1 aa	0.200	566	378	3,817	0.09621	0.55010	22.80670	0.30069	3.474
6	m2 aa	0.102	788	514	3,846	0.09011	0.54063	22.74900	0.30518	3.497
7	m2 aa	0.100	587	379	4,202	0.08678	0.53750	22.18200	0.29931	3.467
BGXM										
8	d-1 aa	0.040	617	386	4,608	0.07806	0.49815	25.44530	0.37046	3.793
9	+100 aa‡	0.250	1,647	722	2,770	0.03040	0.38769	14.15650	0.26483	3.276
10	d-1 aa	0.379	666	441	5,376	0.06491	0.55297	24.25230	0.31809	3.561
11	d-1 aa	0.140	582	477	5,102	0.07294	0.67091	31.20960	0.33738	3.651
12	nm10 aa	0.328	422	399	5,025	0.06974	0.75141	39.63650	0.38258	3.842
13	+100 aa	0.486	699	530	5,263	0.05457	0.62346	29.95230	0.34843	3.700
14	+100 aa	0.161	440	321	3,279	0.07208	0.59430	27.89840	0.34046	3.665
15	nm6	0.284	432	388	3,448	0.08477	0.71299	36.01910	0.36639	3.777
16	+100 aa	0.089	222	218	3,953	0.09161	0.77265	40.58542	0.38097	3.836
17	+100 aa	0.033	613	466	2,451	0.05470	0.62244	29.82996	0.34758	3.697
18	+100 aa	0.027	439	365	2,381	0.05853	0.67586	33.32776	0.35764	3.740
19	feldspar				14.957	2.24200			1.02224	
(b) Sm-Nd										
Sample§	Weight (mg)	Sm (p.p.m.)	Nd (p.p.m.)	¹⁴⁷ Sm/ ¹⁴⁴ Nd	ε ₁₄₃ (0)	ε ₁₄₃ (3.7)	T* _{CHUR} (Gyr)			
BGXM	61	1.53	8.53	0.10817	-47.0 ± 0.3	-4.8	4.10			
BGXM-M	51	2.67	15.62	0.10345	-47.1 ± 0.3	-2.6	3.92			

nm, nonmagnetic; m, magnetic; d, diamagnetic; number following refers to degrees of tilt on the Frantz isodynamic separator; +100 signifies grains larger than 100 mesh size (~240 µm); aa, air-abraded.

* Zircons were separated using standard techniques, dissolved in teflon micro-bombs spiked with a mixed ^{208}Pb - ^{235}U tracer solution, and analysed in single-collector mode using the VG-354 mass spectrometer. Radiogenic ^{206}Pb and ^{207}Pb were calculated by correcting measured ratios with measured blank Pb and with Stacey and Kramers model²⁶ Pb corresponding to the age of the zircons for the original non-radiogenic component. During the course of analysis blanks were <20 pg ^{206}Pb and <15 pg total U. Uncertainties on the $^{207}\text{Pb}/^{206}\text{Pb}$ and U-Pb determinations are estimated from the long-term reproducibility of standards, and samples and are estimated to be ±0.1% for $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ at the 2σ level and ±0.4% for $^{235}\text{U}/^{207}\text{Pb}$ and $^{206}\text{Pb}/^{238}\text{U}$. Hand-picked plagioclase feldspar samples were leached in a stepwise process in order to obtain the least radiogenic Pb. The least radiogenic leach is interpreted as the best approximation of the initial Pb isotopic composition of the sample.

† Zircon fractions analysed at Kansas University.

‡ Discordant fraction not plotted.

§ Powdered samples were totally spiked and dissolved in a HF-HNO₃ mixture in a pressurized teflon capsule at 180 °C for five days; procedures for chemical processing and isotope analysis are as described previously²⁷.

|| Determined by mixed ^{149}Sm and ^{150}Nd spike calibrated against CIT (ref. 28) mixed normal; uncertainty 0.1%.

¶ $^{143}\text{Nd}/^{144}\text{Nd}$ cited as parts in 10⁴ deviation ($\epsilon_{\text{Nd}} = ((I_{\text{S}}/I_{\text{BE}}) - 1) \times 10^4$ where I is the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio, S refers to the sample, BE refers to the bulk Earth) from reference reservoir CHUR²⁸ defined by present $^{143}\text{Nd}/^{144}\text{Nd} = 0.512638$ (for discrimination corrections by $^{146}\text{Nd}/^{144}\text{Nd} = 0.7219$) and $^{147}\text{Sm}/^{144}\text{Nd} = 0.1966$; measured values are adjusted to be consistent with nominal $\epsilon_{\text{Nd}} = -15.2$ for La Jolla standard Nd; errors (2σ) are reproducibility of replicate analyses.

* Time (Gyr) at which $\epsilon(T) = 0$, assuming no change in Sm/Nd.

The Slave Province of north-western Canada yields evidence for the oldest rocks in North America and possibly on Earth. The data reported here extend the occurrence of pre-3.5-Gyr crust to all of the major cratons of North America. The $\epsilon_{\text{Nd}}(3.7)$ of -4.8 indicates that this is one of the few areas on Earth where strongly enriched Archaean crust is preserved, and, if interpreted in the context of existing isotopic models, the data presented here require the involvement of material at least as old as 4.1 Gyr.

An important question concerning the Earth's evolution is whether or not continental crust is the isotopically enriched reservoir that is complementary to the depleted mantle^{6,24}. The tonalite gneisses from the Slave Province represent the first indication of an ancient strongly enriched reservoir (relative to the depleted mantle at 3.8 Gyr) that has been inferred and predicted by others². The data presented here, in conjunction with previous isotope studies of Archaean terranes, support but do not require that large volumes of enriched crust existed earlier than 3.8 Gyr ago. Whether this very ancient remnant from the

Slave Province is representative of Earth's oldest crust or whether its preservation indicates an anomalous composition remains unanswered. The paucity of preserved crust older than 3.8 Gyr is consistent with the development of an early, thick, basaltic crust²⁵ whose formation created a depleted mantle but which was recycled by subduction, giving rise to oceanic arcs. These arcs may then have been amalgamated, thickening to form the earliest continental crust whilst being large enough to be preserved individually as cratonic masses by 3.8 Gyr. Further study of Archaean cratons worldwide should reveal rocks of similar age, providing more constraints on models of the first 700 million years of the Earth's geological history. □

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A primitive seed-like structure and its implications for early gymnosperm evolution

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THE ecological diversity and success of seed plants is generally attributed to their reproductive system. But the origin of the seed habit or gymnospermous reproduction is still in discussion¹, and the question of seed plant monophyly or polyphyly is a matter of considerable debate²⁻⁹. The unique anatomically preserved seed-like structure found in the early Carboniferous, Montagne Noire, France, shows the unequivocal absence of a pollen chamber indicating a more primitive organization than that known from any other late Devonian or early Carboniferous seed. We suggest that the unmodified megasporangial apex may have persisted in this plant under wet ecological conditions, as indicated by anatomical features of the integument lobes. The plant organ represents new fossil evidence of an evolutionary stage between pteridophytic and gymnospermous reproduction and could be interpreted as a very primitive gymnosperm which contradicts current theories of monophyletic gymnosperm origins^{3,4,6,7,9}.

The fossil flora from the Lydiennes Formation, Montagne Noire, southern France is older than the classic late Tournaisian floras of Scotland which have provided the most numerous reports of permineralized Lower Carboniferous seeds¹⁰. Within the Lydiennes Formation, intercalated layers of argillites have

yielded a conodont assemblage indicating a Middle Tournaisian age¹¹. The flora is dominated by vegetative remains of calamopityacean pteridosperms and it is only recently that compressions of reproductive structures characteristic of gymnosperms have been recognized¹¹. Continued searching for material in the cherts of the Lydiennes Formation has now resulted in the discovery of the first anatomically preserved seed-like structure from Coumiac near Causses et Veyran, Hérault, France. The plant organ is described as an integumented, indehiscent megasporangium or nucellus, bearing one functional megaspore and an endosporic gametophyte. This conforms to current definitions of an ovule^{4,12}.

The integument is bell-shaped, 6.5-mm long, 5-mm wide distally, 3.4-mm wide proximally and has radial symmetry (Fig. 1). It is composed of eight lobes (Fig. 2a) which are free from the megasporangium (Fig. 2b). The lobes are fused only at the base and are quadrangular to circular in cross section. They are nearly contiguous and recurved distally where they converge over the sporangium apex (Fig. 1). The anatomy of the integument lobes is characteristic (Fig. 3c) and comprises: (1) an epidermis consisting of small cells constituting the smooth outer surface of the lobe, and intermingling hairs characterizing the epidermis of the inner and lateral lobe surfaces; (2) a hypodermal parenchyma with scattered secretory cells on the outer side of the lobe; (3) an inner spongy parenchyma or aerenchyma; (4) a central vascular strand.

The megasporangium (Fig. 1) is flask-shaped, 3.6-mm long and 1.6-mm in diameter, entirely free, with a massive apical beak, 0.5-mm long and in diameter (Fig. 3b). The megasporangium wall is uniformly thick (250-300 µm) with several layers of thin-walled cells and an epidermis of radially elongated cells with dark contents (Fig. 3a). This configuration resembles that of the Elkins seeds from the Famennian of North America¹³. There is no indication of a differentiated dehiscence zone. The nucellar tip is composed of the same tissue but has smaller epidermal cells (Fig. 3b). There is no pollen chamber. The megasporangium contains an axially elongated megaspore, 2.2-mm long and 0.8-mm in diameter with three aborted

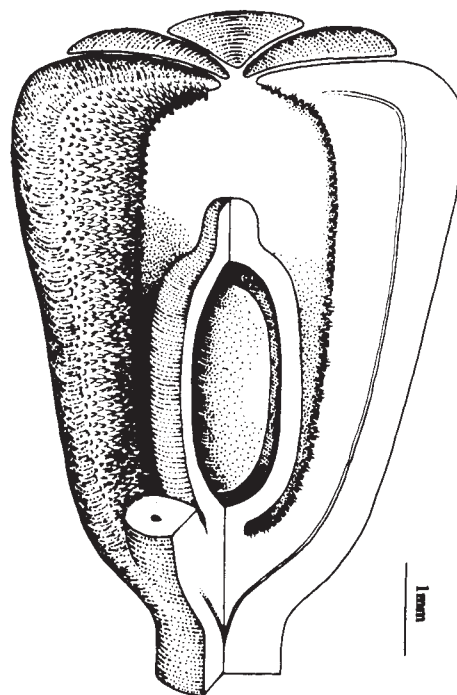


FIG. 1 Reconstruction of the seed-like structure with several integument lobes removed to show the flask-shaped megasporangium and megaspore.

spores at the apex (Fig. 4a). The megaspore membrane is 15–20- μ m thick (Fig. 4b). A gametophyte fills the space inside the megaspore wall (Fig. 2b, 4b). Although individual cells are recognizable, there are no archegonia visible.

Information concerning early seed plants has increased significantly in the past decade and a number of Devonian seeds are now recorded from the Famennian to the Tournaisian^{2,9,13–16}. Although most of these are ovules borne in cupules, we have no evidence of the mode of attachment of the new structure. The Devonian seeds are also smaller (3–7-mm long, 1–2-mm in diameter) than that described here which is up to 5-mm in diameter. A variation is observed among Devonian ovules in the number of integument lobes and their degree of fusion to each other and to the nucellus. The Devonian seed *Moresnetia* shows the least degree of fusion and is considered to have the most primitive organization¹⁴. The new seed has eight unfused lobes and a free nucellus, and therefore most resembles *Moresnetia* and the early Carboniferous seed *Genomosperma*¹⁷. A number of features, however, lead us to distinguish it from all other early seeds, including (1) the occurrence of a massive, parenchyma-filled nucellar tip and the absence of any type of pollen chamber; (2) the thickness of the nucellar wall which exceeds that of the free nucellus in other early seeds; (3) the spatial proximity of the integument lobes which are closely adpressed to each other and are reflexed above the nucellar apex with lateral slits occupied by epidermal hairs; (4) the massive size of the integument lobes (up to 1.2-mm wide) which possess a broad aerenchyma and two sharply differentiated types of epidermis. This is in contrast to the narrow lobes in most early seeds where sclerenchymatous fibres provide mechanical

support. The probability that hypothetical ancestors of the early gymnosperms possessed a simple beak-like nucellar apex which lacked a pollen chamber, has been suggested previously¹⁸. Such an ancestral morphology may have existed among progymnosperms. The new plant organ from the Lydiennes Formation could represent one of the last representatives of such an ancestral group in the early Carboniferous. The new structure does not conform to the supposed archaic 'hydrasperman reproduction' as identified in Devonian and early Carboniferous ovules, which lack a true integumentary micropyle and have a nucellar apex which is modified into an elaborate pollen chamber⁹. The pollen chamber comprises a lagenostome and a prominent central column. It is thus believed to be specialized for wind-borne pollen reception and the post-pollination sealing-off of the sporangium which is supposed to facilitate gametophyte development, fertilization and embryogeny^{9,19}.

It is difficult to speculate on the pollination biology and ontogeny of the new structure from the Lydiennes Formation. Pollen was not found at the apex of the nucellus nor in any part of the integument lobes, which could perhaps be interpreted as evidence of a pre-pollination state. The specimen could also be interpreted as immature so that the breakdown of the nucellar tip to produce a pollen chamber had not yet begun; but this seems less probable considering the differentiation of the other parts of the nucellus and the occurrence of an endosporic gametophyte. Palaeozoic seeds at this stage of development normally have a pollen chamber. In view of the indehiscent nature of the megasporangium and the absence of a pollen chamber, we propose that lysigenous dissolution of the cells in the massive nucellar tip would have operated after pollination

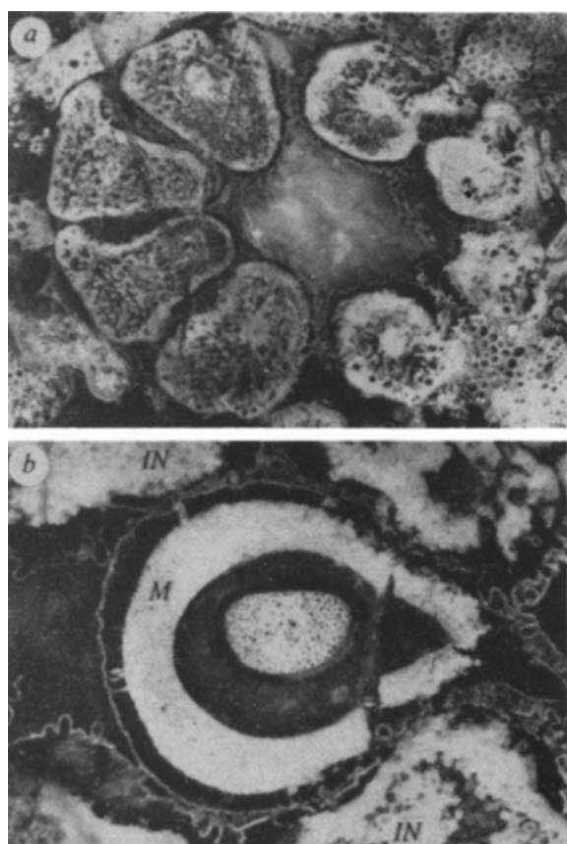


FIG. 2 a, Oblique cross section of the eight integument lobes above the nucellar beak ($\times 12$). b, Section showing four of the integument lobes (IN), the free megasporangium (M) and the megaspore with the white gametophytic tissue. This section is oblique near the top of the megaspore which does not fill the space (dark) of the sporangium cavity ($\times 34$).

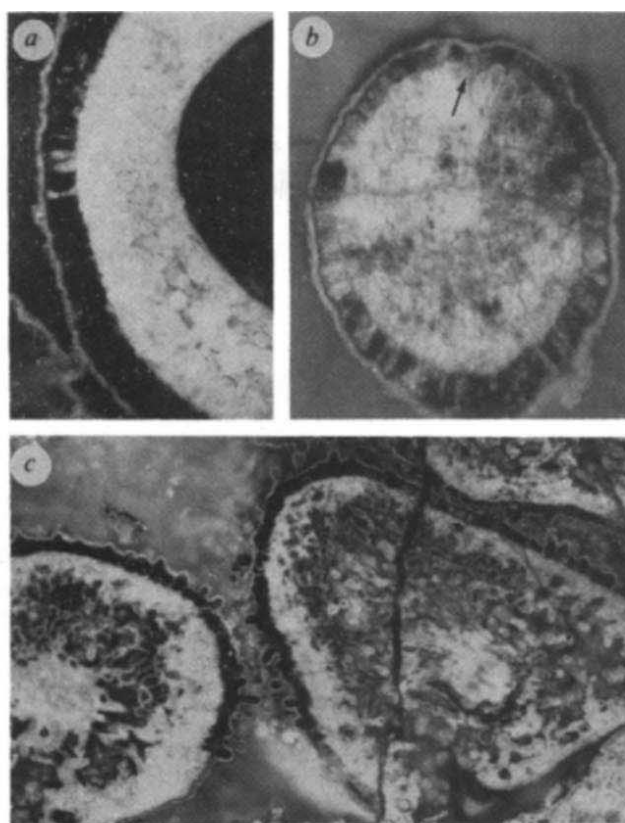


FIG. 3 a, Cross section of the multilayered megasporangium with prominent epidermis ($65\times$). b, Oblique transverse section of the nucellar tip showing inner sterile tissue and epidermis with smaller cells in the region of the apex, arrowed ($65\times$). c, Two integument lobes showing outer smooth epidermis (below), inner epidermis with hairs, aerenchyma and central vascular strand ($30\times$).

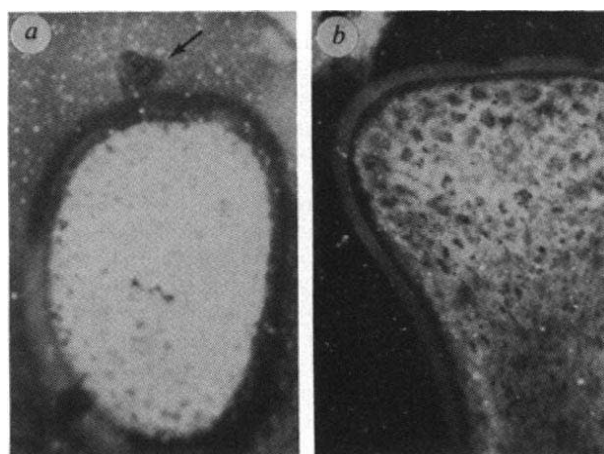


FIG. 4 *a*, Oblique section across the tip of the megaspore showing the three aborted members of the tetrad, arrowed ($\times 100$). *b*, Section in the median region of the megaspore showing the megaspore wall surrounding the gametophytic tissue, the cell wall thickness is a preservation effect ($\times 120$).

and facilitated fertilization. The histology of the broad integument lobes which lack supportive sclerenchyma and have a well developed aerenchyma and a parenchyma with secretory cells, is suggestive of both photosynthetic tissue and an adaptation to an aquatic, marshy or shady habitat.

We propose that the new seed-like structure may have evolved a pollination mechanism which developed under ecological conditions that demanded adaptation for dispersal in water and perhaps water-borne pollination. This would represent a primitive organization where an unmodified megasporangial apex persisted under wet ecological conditions, whereas other gymnosperms evolved an elaborated pollen chamber for the capture of wind-borne pollen.

The monophyly of gymnospermy is founded on hydrasperman reproduction which is widely believed to have characterized all early gymnosperms. The new plant with its integumented megasporangium and massive nucellar beak differs from all the earliest known ovules. It would represent new evidence of an evolutionary stage between pteridophytic and gymnospermous reproduction and could be interpreted as a progymnosperm preovule lacking the pollen chamber which is implicit in the definition of the earliest ovules¹⁹. But we must consider the possibility that this plant is actually a very primitive gymnosperm, which would therefore necessitate a reassessment of the ideas on the monophyletic origin of the group^{3,4,6,7,9}.

Resource availability hypothesis of plant antiherbivore defence tested in a South African savanna ecosystem

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THERE is considerable variation in the levels of herbivory experienced by woody species in ecosystems as diverse as arctic shrub tundra, boreal forests, tropical rain forests and African savannas¹. An important objective of evolutionary ecology is the formulation of a hypothesis that can explain this variation. One such hypothesis predicts that mature leaves of inherently slowly growing species adapted to resource-limited habitats^{2,3} such as infertile soil⁴ are, because of more effective chemical defences, less preferred as a food source by herbivores than mature leaves of inherently rapidly growing species adapted to productive habitats^{2,3,5-8}. In agreement with this prediction, we found positive correlations between the inherent growth rates of nine South African woody species and kudu and impala preferences for their mature leaves. Thus, the resource availability hypothesis of plant antiherbivore defence^{2,3,5-8} could explain why large African herbivores feed less on woody vegetation of dystrophic savanna-woodlands than on woody vegetation of eutrophic savannas⁹.

The composition of African savanna vegetation¹⁰ and the foraging behaviour of large herbivores in African savannas^{9,11} are correlated with soil fertility. Eutrophic *Acacia* savanna is characterized by palatable woody species, whereas dystrophic savanna-woodland is dominated by unpalatable species such as *Burkea africana*. For our experiment we selected nine species (Table 1) that are common in the *Acacia* savanna and *Burkea* woodland of our study site, the Nylsvley Nature Reserve, northern Transvaal, South Africa¹². At the time of our experiment the preferences of two common African ruminants, kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*), for mature leaves of these nine species were known^{13,14}. The inherent growth rates of these species, however, were unknown. Thus, we were able to test the resource availability hypothesis of plant antiherbivore defence^{2,3,5-8} by using known kudu and impala preferences to predict the comparative inherent growth rates of the nine species. We then determined these inherent

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TABLE 1 Occurrence of nine South African savanna woody species in *Acacia* savanna and *Burkea africana* woodland

Species	Abbreviation	Vegetation type	Seedling biomass
<i>Acacia burkei</i>	A	A	7.31 $\pm$ 0.82
<i>Acacia karroo</i>	B	A	16.04 $\pm$ 1.15
<i>Acacia nilotica</i>	C	A	12.91 $\pm$ 1.49
<i>Acacia tortilis</i>	D	A	15.70 $\pm$ 0.82
<i>Dicrostachys cinerea</i>	E	A	16.51 $\pm$ 0.74
<i>Grewia flavescens</i>	F	BA	22.13 $\pm$ 1.15
<i>Peltophorum africanum</i>	G	BA	5.60 $\pm$ 0.61
<i>Burkea africana</i>	H	B	3.39 $\pm$ 0.35
<i>Euclea natalensis</i>	I	B	0.15 $\pm$ 0.01

Acacia savanna, A; *Burkea africana* woodland, B. Seedling biomass is dry biomass of seedlings after three months of growth, presented as mean $\pm$ s.e.m. (Vegetation types are from ref. 14, Table 1a.)

growth rates in a glass house experiment. A positive correlation between the predicted and the measured growth rates supports the resource availability hypothesis of plant antiherbivore defence, whereas either a lack of correlation or a negative correlation between them is sufficient evidence to reject the hypothesis for South African savanna woody plants and large herbivores.

Fifteen individual specimens of each species were grown from seed collected at Nylsvley in sand culture (21 pots) in a glass house at the University of Alaska, Fairbanks for three months (February–April). At this time of year the photoperiod at Fairbanks ranges from 10–14 h. During the experiment the light intensity just above the crowns of the seedlings averaged $1,300 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the temperature was maintained between 24 °C (night) and 30 °C (day). This photoperiod, light and temperature regimen is within the range experienced at mid-growing season by seedlings growing in the field at Nylsvley¹⁵. The pots were flushed every other day with a modified Hoagland's nutrient solution (pH 6.5) containing

70 p.p.m. ammonium nitrogen, 70 p.p.m. nitrate nitrogen, 60 p.p.m. phosphorus as K_2HPO_4 , and micronutrients. Pilot experiments demonstrated that this feeding regimen favoured rapid growth of all nine species. After three months of growth the seedlings were washed free of sand, dried for 48 h at 75 °C, weighed and their dry biomass used to estimate their inherent growth rate¹⁶.

Burkea woodland species grew more slowly than *Acacia* savanna species with one exception, *Grewia flavescens* (Table 1). This species, however, commonly occurs on termitaria¹⁷, nutrient enriched microhabitats within savanna soils^{18–21}. Thus, the high inherent growth rate of *G. flavescens* probably reflects adaptation to fertile soil. These results are in agreement with the generalization that plant species characteristic of infertile soil have lower inherent growth rates than do species characteristic of fertile soil^{2–4}. Results presented in Fig. 1 demonstrate statistically significant positive correlations between the seedling biomasses of the nine species we studied and field measurements of kudu and impala preferences for mature leaves.

Other studies have established that large African herbivores feed less on vegetation characteristic of infertile soil than on vegetation characteristic of fertile soil^{9,11}. Bell⁹ suggested that this widespread relationship between soil fertility, vegetation and large herbivore foraging in African savannas is causal: vegetation growing on infertile soil is comparatively poor food for large herbivores, because it has a low ratio of nutrients/(fibre + chemical defences). This suggestion, which has recently gained support^{13,14}, is consistent with expectations of the resource availability hypothesis of plant antiherbivore defence and may provide a general theoretical framework for interpreting interactions between large African herbivores and their food resources.

Throughout African savannas the demand for food by a rapidly growing human population is resulting in increased use of fertile soil for crop production. Consequently, livestock and wildlife are increasingly being forced into habitats that are unsuitable for agriculture, because of their infertile soil²². The resource availability hypothesis of plant antiherbivore defence predicts two consequences of this change in land use. First, increased use of vegetation in infertile soil as forage will result in decreased production of large herbivore biomass because of forced consumption of unproductive, chemically defended woody vegetation. Second, increased browsing of slowly growing woody species by large herbivores will result in increasingly severe vegetation degradation, because inherently slowly growing plant species have, in comparison to fast growing species, a low capacity for regrowth. If unchecked, such degradation of vegetation will result in either domination of the vegetation by species that are so chemically defended as to be inedible, or denudation, erosion and ultimately desertification. Thus, we are in agreement with Bell's proposal²³ that intensive studies of interactions between soil fertility, the defensive chemistry and regrowth capacity of vegetation, and the foraging behaviour of large herbivores are essential for ecologically sound management of African savannas. □

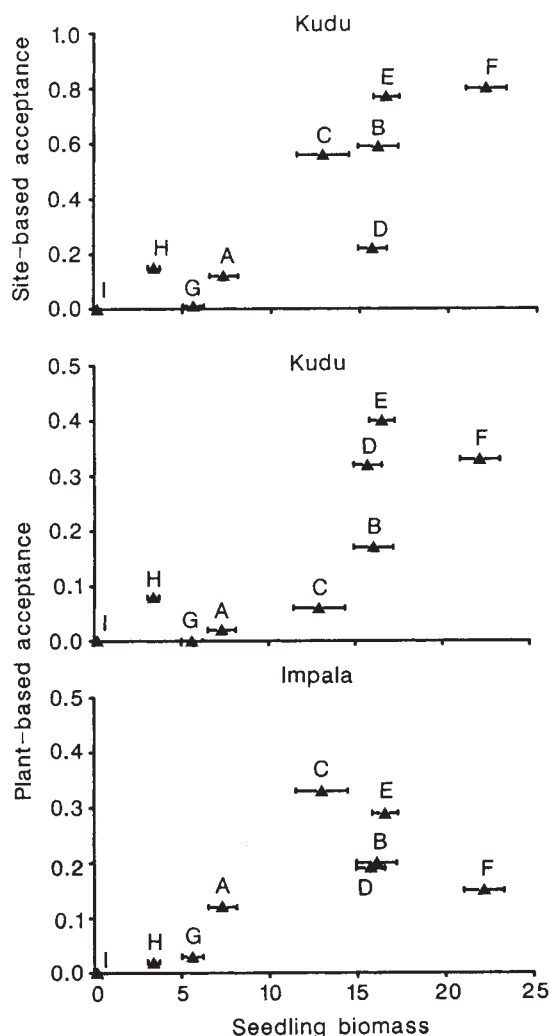


FIG. 1 Relationships between the seedling dry biomasses of nine South African savanna woody species and field measurements of kudu (a, b) and impala (c) preferences for their mature leaves. Abbreviations of plant scientific names are presented in Table 1. Kudu and impala preferences are acceptance values from ref. 14, Table 1a. Seedling dry biomasses were determined in a glass-house experiment, as described in the text. Seedling biomasses are presented as mean $\pm$ s.e.m. Spearman rank correlations, r_s , were used to measure the significance of the correlations between seedling-biomass means and acceptance values. a, $r_s = 0.933$, $P = 0.0005$; b, $r_s = 0.858$, $P < 0.005$; c, $r_s = 0.753$, $P < 0.025$.

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Limits on bilingualism

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SPEECH, in any language, is continuous; speakers provide few reliable cues to the boundaries of words, phrases, or other meaningful units. To understand speech, listeners must divide the continuous speech stream into portions that correspond to such units. This segmentation process is so basic to human language comprehension that psycholinguists long assumed that all speakers would do it in the same way. In previous research^{1,2}, however, we reported that segmentation routines can be language-specific: speakers of French process spoken words syllable by syllable, but speakers of English do not. French has relatively clear syllable boundaries and syllable-based timing patterns, whereas English has relatively unclear syllable boundaries and stress-based timing; thus syllabic segmentation would work more efficiently in the comprehension of French than in the comprehension of English. Our present study suggests that at this level of language processing, there are limits to bilingualism: a bilingual speaker has one and only one basic language.

As in our earlier experiments, subjects listen to lists of unrelated words and press a response key as soon as they hear a word beginning with a specified word-initial sequence of sounds,

which is either a consonant–vowel (CV, for example, *ba-*) or a consonant–vowel–consonant (CVC, for example, *bal-*). French speakers listening to French responded faster when the target sequence was exactly the initial syllable of a word than when it was more or less of the word than the initial syllable. For instance, their responses to *ba-* were faster in *balance* (first syllable *ba*) than in *balcon* (first syllable *bal*), but *bal-* responses were faster in *balcon* than in *balance* (Fig. 1a).

English listeners performing the same task on English words such as *balance* and *balcony*, however, did not respond differently to CV and CVC targets in either word type (Fig. 1b). When English listeners heard the French words, they still showed no sign of syllable-based responding (Fig. 1d); but French listeners' responses to the English words were as syllable-based as their responses to French words had been (Fig. 1c).

We concluded that speakers use syllabic segmentation only if their native language encourages it. Those who first acquired French, in which syllabic segmentation is efficient, can use syllabification even when they are listening to other languages. Those who first acquired English, in which syllabic segmentation is inefficient, cannot syllabify even when they are listening to a language, like French, which encourages syllabification.

In the present study we asked: can speakers who command two languages perfectly also vary their segmentation routines? We tested speakers who acquired two languages, French and English, in early childhood, still spoke both languages regularly, and were accepted by other native speakers of each language as native speakers; furthermore, 75% of our speakers had one native French-speaking and one native English-speaking parent.

We tested 13 such subjects in England and 14 in France. The subjects were required to express a preference for one of their languages. Although they all averred that they spoke each language with equal ease, they were made to provide an answer to the question: 'if you had to lose one of your languages to save your life, which would you keep?' This answer we termed their 'dominant' language. Each subject performed both the French and the English experiment from our previous studies with monolinguals.

When the group of 27 subjects was considered as a whole, the results with each set of materials did not resemble the performance of the monolingual groups in our previous studies. When our subjects were divided by choice of dominant language, however (15 English, 12 French), their results showed a pattern which can be related to our previous findings. English-dominant subjects performed like English monolinguals with both the

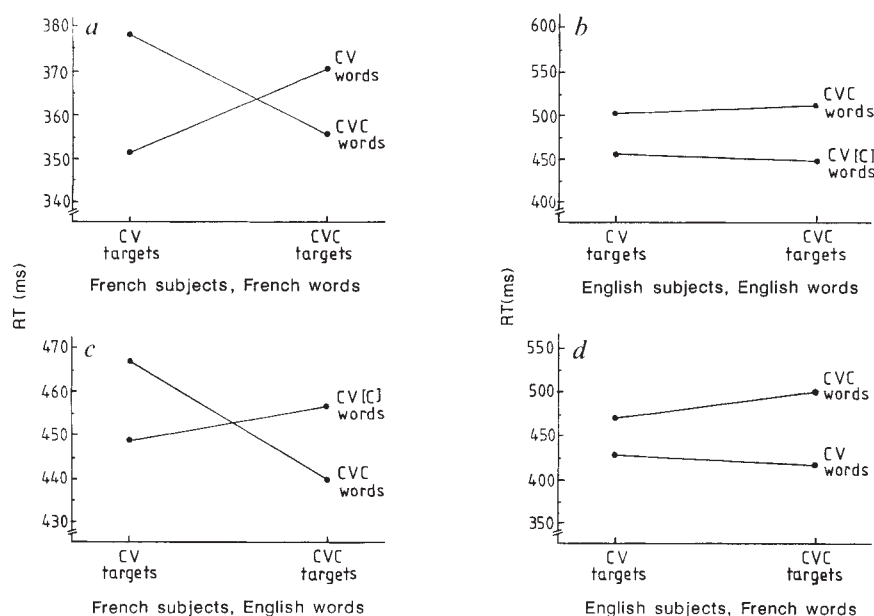


FIG. 1 Mean target-detection response time (RT) as a function of size of target sequence (CV, for example, *ba-*, versus CVC, for example, *bal-*) and size of initial syllable of stimulus word (CV versus CVC for French; CV[C] versus CVC for English), for four combinations (a–d) of subjects' native language and stimulus presentation language.

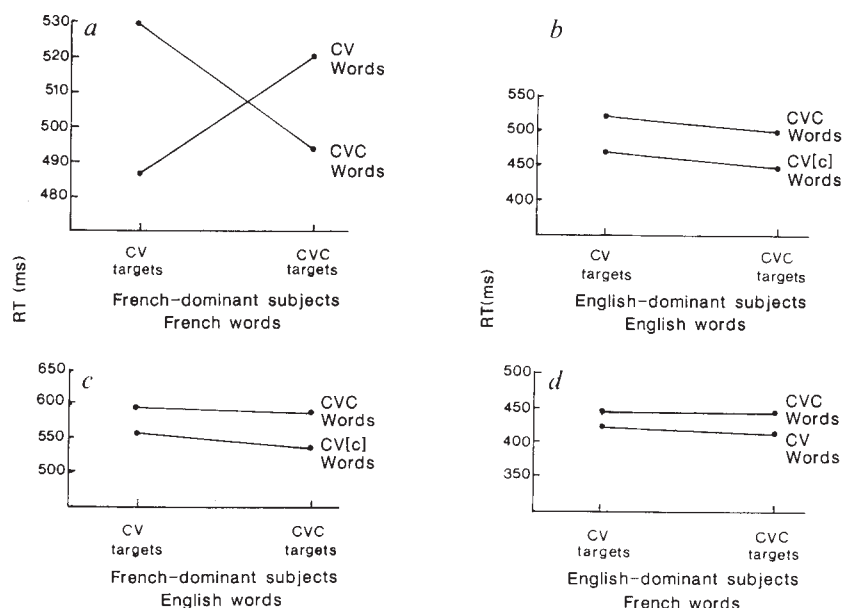


FIG. 2 Mean target-detection response time (RT) as a function of size of target sequence (CV, for example, *ba-*, versus CVC, for example, *bal-*) and size of initial syllable of stimulus word (CV versus CVC for French; CV[C] versus CVC for English), for four combinations (a-d) of subjects' dominant language and stimulus presentation language.

English and the French materials (Figs. 2b and 2d). French-dominant subjects behaved like French monolinguals with the French materials (Fig. 2a) and like English monolinguals with the English materials (Fig. 2c).

These results suggest several conclusions of considerable theoretical import. First, the fact that the group as a whole did not perform like either monolingual group is evidence that even speakers who by any pragmatic definition command two languages perfectly do not necessarily function as two monolinguals in one person. Instead, for any speaker, one and only one language is basic. Bilinguals who were English-dominant did not use syllabic segmentation when listening to either of their native languages; the only bilinguals who used syllabic segmentation were those who were French-dominant. Language dominance fully determined the speech segmentation routines that our subjects could apply.

It is notable, however, that the French-dominant bilinguals did not use syllabic segmentation when they were listening to English. As we have argued, syllabic segmentation is inefficient for processing English, but French monolinguals, as our previous studies showed, have no option but to use it. French-dominant French-English bilinguals, however, seem to be able to abandon syllabic segmentation when it would be inefficient. This prompts our second main conclusion: that syllabic segmentation is a special ('marked') language processing routine which speakers develop and apply only if their (dominant) native language encourages it. The majority of languages, including English, do not encourage it; their speakers develop only 'unmarked' non-syllabic routines (which may work adequately on any language). A speaker who segments syllabically can, it seems, with sufficient exposure to cases in which this is inefficient, also develop the unmarked process. Speakers who begin with unmarked routines, however, can apparently not develop syllabic segmentation, no matter how extensive their exposure to cases in which it would be more efficient. Therefore the English-dominant bilinguals necessarily performed like English monolinguals with both languages, whereas the only bilinguals who showed evidence of both syllabic and non-syllabic segmentation were those who were French-dominant. Thus, a speaker can simultaneously command a marked and an unmarked segmentation routine only when the language which encourages use of the marked routine dominates the language which encourages use of the unmarked routine. This finding has obvious bearing on contemporary parameter-setting theories of language acquisition³. □

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Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel

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IN cardiac muscle, where Ca^{2+} influx across the sarcolemma is essential for contraction, the dihydropyridine (DHP)-sensitive L-type calcium channel¹ represents the major entry pathway of extracellular Ca^{2+} . We have previously elucidated the primary structure of the rabbit skeletal muscle DHP receptor by cloning and sequencing the complementary DNA². An expression plasmid carrying this cDNA, microinjected into cultured skeletal muscle cells from mice with muscular dysgenesis, has been shown to restore both excitation-contraction coupling and slow calcium current missing from these cells, so that a dual role for the DHP receptor in skeletal muscle transverse tubules is suggested³. We report here the complete amino-acid sequence of the rabbit cardiac DHP receptor, deduced from the cDNA sequence. We also show that messenger RNA derived from the cardiac DHP receptor cDNA is sufficient to direct the formation of a functional DHP-sensitive calcium channel in *Xenopus* oocytes. Furthermore, higher calcium-channel activity is observed when mRNA specific for the polypeptide of relative molecular mass $\sim 140,000$ (α_2 -subunit)⁴⁻⁶ associated with the skeletal muscle DHP receptor is co-injected.

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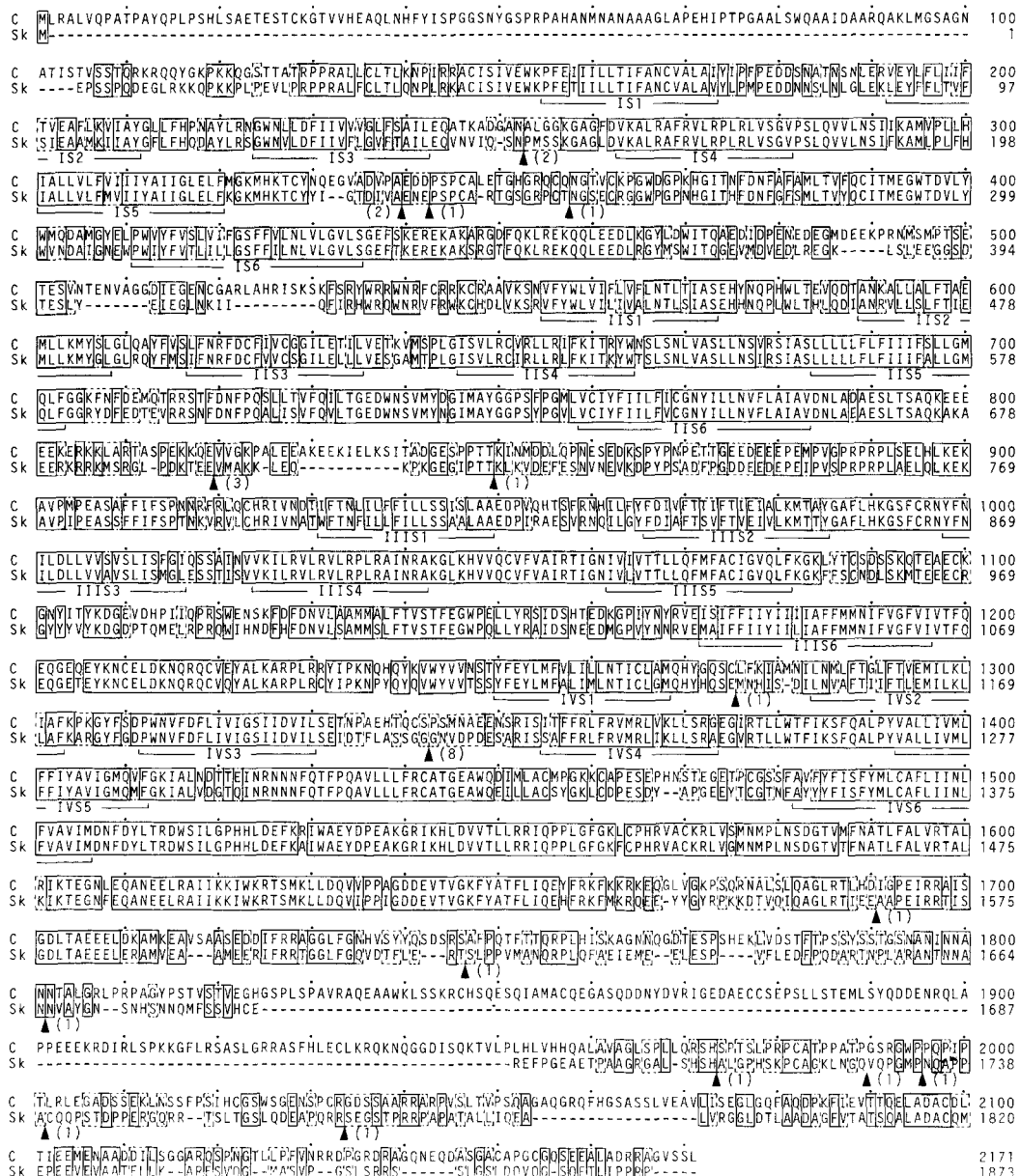
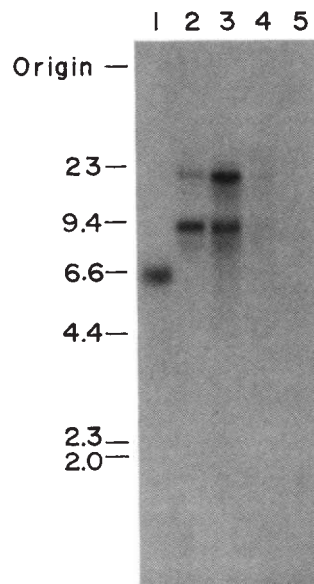


FIG. 1 Alignment of the amino-acid sequence (C, top) of the rabbit cardiac DHP receptor (as predicted from the cloned cDNA; the 7,036-nucleotide sequence to be deposited in a databank, see end of main text) with that of the rabbit skeletal muscle DHP receptor² (Sk, bottom). For numbering of the amino-acid residues, see ref. 7. Numbers of the amino-acid residues at the right-hand end of the individual lines are given. Sets of identical amino-acid residues are enclosed with solid lines, and sets of conservative residues⁷ with broken lines. Deletions and insertions in the amino-acid sequence of the skeletal muscle DHP receptor, as compared with that of the cardiac DHP receptor, are indicated by gaps (—) and triangles (with the number of inserted residues in parentheses), respectively. The putative transmembrane segments S1–S6 in each of the repeats I–IV are shown; the termini of each segment are tentatively assigned by comparison with the skeletal muscle DHP receptor². In the cDNA sequence (not shown, see above), nucleotide 6,845 is followed by a stretch of nine dA residues, which may not represent the poly(A) tail because no conventional polyadenylation signal is found; nucleotide residues are numbered from the first residue of the ATG initiation triplet and the preceding residues are indicated by negative numbers. METHODS. A randomly primed cDNA library was constructed²² in plasmid pBR322 using poly(A)⁺ RNA prepared⁸ from adult rabbit heart. It was screened with a rabbit skeletal muscle DHP receptor cDNA probe, that is, an equimolar mixture of the *Apal*(804)–*Apal*(3098), *Apal*(3098)–*Apal*(4187)

and *Apal*(4187)–*Apal*(5131) fragments from pCCH102 (ref. 2); restriction endonuclease sites are identified by numbers (in parentheses) indicating the 5'-terminal nucleotide generated by cleavage. Thus clone pCCAR2 (3,194–3,488) was isolated; numbers in parentheses indicate the nucleotide residues of the cDNA carried by the clone. Another randomly primed cDNA library was constructed in phage λ gt10. Double-stranded cDNA prepared using the cDNA synthesis system (Bethesda Research Laboratories) was blunted by T4 DNA polymerase, methylated by *Eco*RI methylase, ligated with an *Eco*RI linker and cleaved by *Eco*RI. Size-selected fragments longer than ~1 kilobase pair (kb), prepared by electrophoresis on 1% agarose gel, were ligated with λ gt10. Clone λ CCAR1 (2,220–4,006) was isolated by hybridization with the *Hinf*I (3231)–*Hinf*I (3467) fragment from pCCAR2, and clone λ CCAR34 (–191 to 2,212) with the *Apal*(804)–*Apal*(3,098) fragment from pCCH102. An oligo(dT)-primed, size-selected (< ~3 kb) cDNA library, constructed in λ gt10 as described above, was screened with the 3.1 kb *Bal*I (3800)–*Eco*RI (linker) fragment derived from λ CCAR1 to yield clone λ CCAR105 (554–6,845 and the stretch of nine dA residues). Appropriate restriction fragments from λ CCAR1, λ CCAR34 and λ CCAR105 were subcloned into pBluescript (Stratagene) or M13mp18. Nested deletions were made²³ and DNA sequencing was carried out on both strands by the dideoxy chain termination method²⁴. Gaps or ambiguities were resolved by chemical sequencing²⁵ of some fragments.

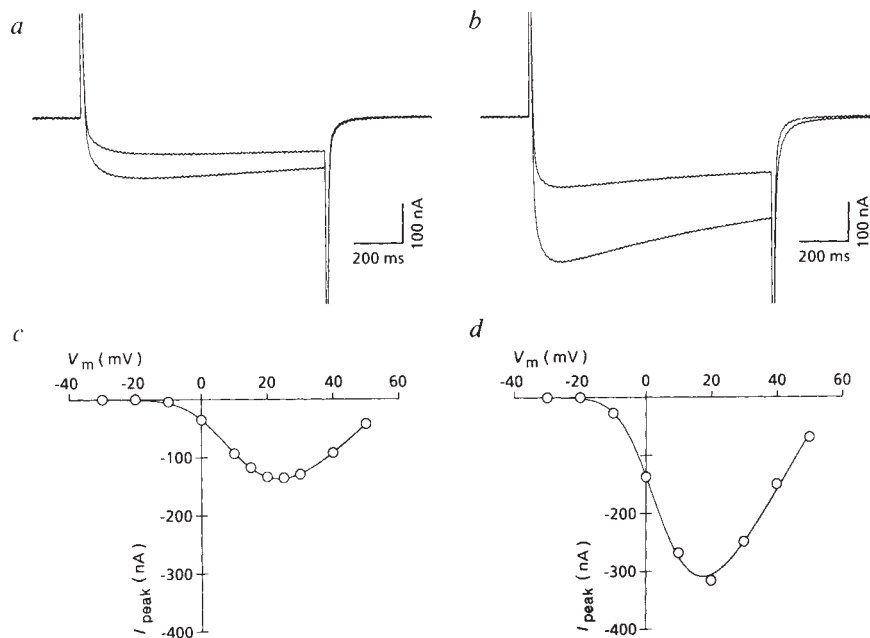
Figure 1 shows the amino-acid sequence of the rabbit cardiac DHP receptor as predicted from the cloned cDNA (for cloning procedure, see legend to Fig. 1). The primary structure of this protein was deduced using the open reading frame corresponding to the amino-acid sequence of the skeletal muscle DHP

FIG. 2 Autoradiograph of blot hybridization analysis of poly(A)⁺ RNA from different adult rabbit tissues with a cardiac DHP receptor cDNA probe. Poly(A)⁺ RNA prepared⁸ from skeletal muscle (10 µg, lane 1), heart (3 µg, lane 2), brain (10 µg, lane 3), stomach (10 µg, lane 4) and kidney (10 µg, lane 5) was analysed as described previously²⁶, except that 1.2% agarose gel was used and the filter was washed with 0.3×SSC containing 0.1% SDS. The probe used was the *Eco*RI (2,215)–*Eco*RV (4,351) fragment derived from λCCAR105 and labelled by nick translation. Autoradiography was at –70 °C for 7 days with an intensifying screen. The size markers used were the *Hind*III cleavage products of phage λ DNA (sizes in kb).



receptor. The translational initiation site was assigned to the first ATG triplet that appears downstream of nonsense codons found in-frame. The rabbit cardiac DHP receptor is composed of 2,171 amino acids, having a relative molecular mass (M_r) calculated as 242,771. The cardiac DHP receptor, like its skeletal muscle counterpart² and voltage-dependent sodium channels^{7–10}, contains four repeated units of homology (amino-acid residues 141–438, 540–786, 917–1,199 and 1,236–1,509). Each repeat has five hydrophobic segments (S1, S2, S3, S5 and S6) and one positively charged segment (S4) (refs 2, 7–10). The conserved charged residues in segments S2 and S3 (refs 2, 7–10) are also retained. The degree of amino-acid sequence homology between the cardiac and the skeletal muscle DHP receptor is 66%. The regions corresponding to the four internal repeats are highly conserved, whereas the remaining regions, all of which are assigned to the cytoplasmic side of the membrane, are less well conserved, except for the short segment between repeats III and IV. Both the amino-terminal and the carboxy-terminal region of the cardiac DHP receptor are larger than those of the skeletal muscle DHP receptor. The structural similarity observed suggests that the cardiac DHP receptor has the same transmembrane topology proposed for its skeletal muscle counterpart² and voltage-dependent sodium channels^{7–10}. This model is consistent with four of the thirteen potential *N*-glycosylation sites¹¹ (Asn residues 183, 358, 1,418 and 1,469) being located on the extracellular side and with six of the seven potential cyclic AMP-dependent phosphorylation sites¹² (Ser residues 124, 1,575, 1,627, 1,700, 1,848 and 1,928) on the cytoplasmic side; residues 183 (with a shift by one residue) and 358 and residues 1,627 and 1,700 are conserved in the skeletal muscle DHP receptor.

FIG. 3 Properties of I_{Ba} in *Xenopus* oocytes injected with mRNA specific for the cardiac DHP receptor alone (a, c) or in combination with mRNA specific for the skeletal muscle 140K polypeptide (b, d). a and b, I_{Ba} recorded with a two-microelectrode voltage clamp. The inward currents were elicited by a 1.0 s pulse stepped from a holding potential of –50 mV to +10 mV before (upper trace) and during exposure to 5 µM BAY K 8644 (lower trace). The increase in peak current caused by 5 µM BAY K 8644 was variable (1.7- to 10-fold) in different oocytes. c and d, Peak current (I_{peak}) plotted against test potential (V_m). I_{Ba} was measured in the absence of BAY K 8644 and the record obtained in the presence of 0.2 mM CdCl₂ was subtracted. Our detectable limit was ~5 nA. METHODS. The pSP72 (Promega) recombinant plasmid pCARD3 carrying the entire protein-coding sequence of the rabbit cardiac DHP receptor cDNA was constructed as follows. The cDNA inserts of λCCAR34 and λCCAR105 were subcloned into the *Eco*RI site of pBluescript KS to yield pCCAR34 and pCCAR105, respectively. *Sma*I-cleaved pCCAR34 was ligated with a *Hind*III linker and cleaved by *Hind*III and *Stu*I. *Xba*I-cleaved pCCAR105 was blunted by Klenow fragment, ligated with a *Hind*III linker and cleaved by *Hind*III and *Stu*I. The resulting 2.0-kb *Hind*III–*Stu*I fragment and 5.0-kb *Stu*I–*Hind*III fragment were ligated with *Hind*III-cleaved pKCRH2 (ref. 27) to yield pCARD1. The 6.8-kb *Hind*III–*Hpa*I fragment from pCARD1, the ~0.31-kb *Nae*I–*Eco*RI fragment (carrying a poly(dA)·poly(dT) tract) from pSPBM1 (ref. 28) and the 3.0-kb *Eco*RI–*Hind*III fragment from pBluescript KS were ligated to yield pCARD2. The 7.2-kb *Hind*III–*Xba*I fragment from pCARD2 was ligated with the 2.4-kb *Xba*I–*Hind*III fragment from pSP72 to yield pCARD3. cDNA encoding the rabbit skeletal muscle 140K polypeptide was cloned by procedures similar to those described previously². The deduced amino-acid sequence of the 140K polypeptide is the same as that reported recently²⁹, except that the His residue 31 is replaced by Asn and Ser 594 is deleted. The pSP65 (Promega) recombinant plasmid pSPCA1 carrying the entire protein-coding sequence of the rabbit skeletal muscle 140K polypeptide cDNA (~7.5-kb extending from the *Fnu*DII site located 5 base pairs upstream from the translational initiation codon to the *Pvu*II site on the Okayama–Berg



vector) was constructed. The mRNA specific for the cardiac DHP receptor and that for the skeletal muscle 140K polypeptide were synthesized *in vitro*¹⁶ using *Xba*I-cleaved pCARD3 and *Sal*I-cleaved pSPCA1, respectively, as templates. *Xenopus laevis* oocytes were injected with the DHP receptor-specific mRNA (0.3 µg µl⁻¹) alone or in combination with the 140K polypeptide-specific mRNA (0.3 µg µl⁻¹); the average volume injected was ~50 nl per oocyte. The injected oocytes were incubated at 19 °C for 2–3 days in modified Barth's medium³⁰ containing cefmenoxime (0.1 mg ml⁻¹) and mycostatin (15 µg ml⁻¹). The follicular cell layer was removed³⁰ from oocytes before electrophysiological measurements. Whole-cell currents were recorded³⁰ at 20 ± 2 °C in a solution of the following composition¹³ (in mM): 40 Ba²⁺, 50 Na⁺, 2 K⁺, 5 HEPES (pH 7.4 with methanesulphonic acid). Current records were sampled at 1.6 ms intervals after low-pass filtering at 30 Hz.

Poly(A)⁺ RNA preparations from different rabbit tissues were subjected to blot hybridization analysis with a cardiac DHP receptor cDNA probe (Fig. 2). The heart contains two major hybridizable RNA species of ~8,900 and ~15,500 nucleotides, the former exhibiting a more intense signal than the latter (lane 2). The probe also detects the skeletal muscle DHP receptor mRNA of ~6,500 nucleotides, which was described previously² (lane 1). Both the brain (lane 3) and the stomach (lane 4) contain two major hybridizable RNA species with sizes similar to those of the cardiac RNAs. No hybridizable RNA is detected in the kidney (lane 5). The nature of the different RNA species observed in the heart, brain and stomach is not known.

Messenger RNA specific for the cardiac DHP receptor was synthesized by transcription *in vitro* of the cloned cDNA and was injected into *Xenopus* oocytes. In normal saline with 1.8 mM Ca²⁺ (ref. 13), injected oocytes showed a transient outward current when the membrane potential was stepped from -50 mV to +10 mV under voltage clamp. Such a transient outward current has previously been observed in *Xenopus* oocytes injected with rat heart RNA and has been shown to be a current through an endogenous chloride channel activated by Ca²⁺ entering the cell¹³. To resolve the calcium current, we replaced external Ca²⁺ with Ba²⁺ (40 mM) and external Cl⁻ with methanesulphonate¹³. In this solution, the transient outward current disappeared and a sustained inward current was evoked by depolarizing pulses (Fig. 3a, upper trace); no such current was detected in 26 non-injected oocytes under the experimental conditions used. The inward current was unaffected by addition of tetrodotoxin (30 µM) or by replacement of both Na⁺ and K⁺ with Ba²⁺ (final concentration, 100 mM), but was completely suppressed by addition of Cd²⁺ (0.2 mM). The peak inward current was increased several times by 5 µM BAY K 8644 (Fig. 3a, lower trace), whereas it was decreased to 30–80% of the control value by 1 µM nifedipine and was virtually abolished by 10 µM nifedipine (data not shown). These results, taken together, indicate that the inward current is a barium current (*I*_{Ba}) through a DHP-sensitive calcium channel.

Purified DHP receptor preparations contain an additional large glycosylated polypeptide of *M*_r ~140K under reducing conditions^{4–6}. The possibility has been suggested that this polypeptide, which seems to be highly conserved in different excitable tissues⁶, may be involved in calcium-channel function^{4–6}. We therefore examined *Xenopus* oocytes that were injected with mRNA specific for the rabbit skeletal muscle 140K polypeptide, together with mRNA specific for the cardiac DHP receptor. Figure 3b shows typical recordings of *I*_{Ba} in such an oocyte in the absence (upper trace) and presence (lower trace) of 5 µM BAY K 8644. The peak inward current observed in the absence of BAY K 8644 was 82 ± 70 nA (mean ± s.d., range 10–272 nA, *n* = 29, excluding three unresponsive oocytes). This value was substantially larger than that obtained for oocytes injected only with the DHP receptor-specific mRNA (31 ± 26 nA, range 6–109 nA, *n* = 26, excluding four unresponsive oocytes). But neither the sensitivity to BAY K 8644 (5 µM) and nifedipine (1 µM), nor the peak current-voltage relationship (Fig. 3c, d) was affected appreciably by co-injection of the 140K polypeptide-specific mRNA. Injection of only this mRNA induced no detectable *I*_{Ba} in 25 oocytes.

Our results indicate that the DHP receptor alone is sufficient to exhibit DHP-sensitive calcium channel activity, on the assumption that *Xenopus* oocytes do not contain equivalents to other polypeptides that may associate with it to form the functional channel. This conclusion is in accordance with the previous findings that mRNAs specific for the sodium channel^{14–17} and the potassium channel^{18,19}, which share common structural features with the DHP receptor, can by themselves produce the functional channels in *Xenopus* oocytes. By analogy with the skeletal muscle DHP receptor^{2,3}, it is possible that the cardiac DHP receptor, in addition to its role as the sarcolemmal DHP-sensitive calcium channel, may serve as the voltage sensor to

control the release of Ca²⁺ from the sarcoplasmic reticulum. The mechanism underlying the observed effect of co-injection of the 140K polypeptide-specific mRNA is not known. This protein may increase the level of expression of the DHP receptor by facilitating its localization in the membrane or by altering its stability or may modulate its channel properties. Of interest in this context are the observations that expression of functional (Na⁺ + K⁺)ATPase in *Xenopus* oocytes requires not only the catalytic α -subunit but also the glycoprotein β -subunit²⁰, whereas Ca²⁺-ATPase, expressed in COS-1 cells, shows no such requirement for a glycoprotein²¹.

The sequence of the rabbit cardiac DHP receptor cDNA will appear in the EMBL/GenBank/DBJ Nucleotide Sequence Databases under the accession number X15539. □

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Induction of calcium currents by the expression of the α_1 -subunit of the dihydropyridine receptor from skeletal muscle

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THE dihydropyridine (DHP) receptor purified from skeletal muscle comprises five protein subunits (α_1 , α_2 , β , γ and δ) and produces Ca²⁺ currents that are blocked by DHPs¹. Cloning of the α_1 - and α_2 -subunits^{2,3}, the former affinity-labelled by DHP, has shown that the α_1 -subunit is expressed in skeletal muscle alone, whereas the α_2 - and δ -subunits are also expressed in other

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tissues^{2,4,5}. Although the transient expression of the α_1 -subunit in myoblasts from dysgenic mice (but not in oocytes) has been demonstrated⁶, the use of these expression systems to determine the function of the α_1 -subunit is complicated by the presence of endogenous Ca^{2+} currents^{7,8}, which may reflect the constitutive expression of proteins similar to the α_2 -, β -, γ - and/or δ -subunits. We therefore selected a cell line which has no Ca^{2+} currents or α_2 -subunit, and probably no δ -subunit for stable transformation with complementary DNA of the α_1 -subunit. The transformed cells express DHP-sensitive, voltage-gated Ca^{2+} channels, indicating that the minimum structure of these channels is at most an $\alpha_1\beta\gamma$ complex and possibly an α_1 -subunit alone.

We co-transfected murine L cells with the thymidine kinase selection gene (*tk*) and pDHP α_1 -tot, a plasmid containing the complete open reading frame of the cDNA of the skeletal muscle dihydropyridine receptor (DHP) α_1 -subunit^{2,3} under the control of a metallothionein promoter⁹. RNAs from 7 out of 20 cloned cell lines were positive for DHP α_1 messenger RNA and the three lines giving the most intense signals (Fig. 1a), were expanded further. These showed low but significant specific DHP binding (Fig. 1b), characterized by a K_d of 0.4 nM in LCa. 11 cells and a maximum specific-binding capacity of 50–60 fmol mg⁻¹ membrane protein (data not shown). This contrasts with 500–1,000 fmol per mg of cardiac sarcolemmal membranes and 15,000–80,000 fmol per mg of T-tubule membrane^{10,11}. On average, each LCa. 11 cell expresses 600–1,000 DHP-binding sites. Significantly, *tk*⁻ L cells or LEM5 cells, transfected with the same expression plasmid⁹ but containing the open reading frame of the M5 muscarinic acetylcholine receptor¹¹, showed no DHP binding.

An immunoblot analysis for the presence of α_1 in LCa. 11 cells¹², revealed a band corresponding to a relative molecular

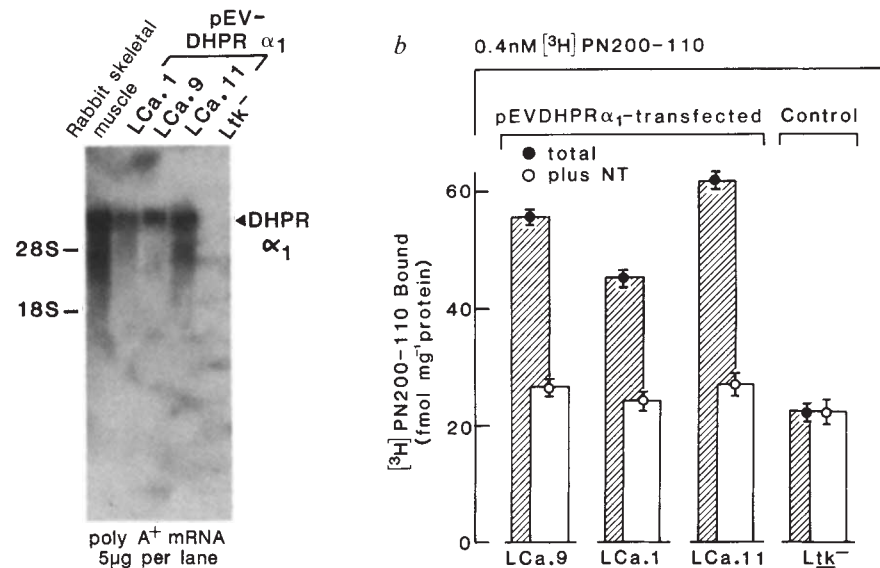
mass (M_r) of 195,000 (195K) (Fig. 2). This is greater than the apparent M_r of the T-tubule α_1 -subunit (170K), indicating that either the L cell processes the α_1 -subunit differently from the skeletal muscle cell, or that during isolation the skeletal muscle α_1 -subunit is proteolytically cleaved. We noted, for instance, that LCa. 11 cell α_1 -subunit is easily cleaved to a major fragment migrating with M_r 170K, raising the possibility¹³ that one of the smaller components is derived from the same precursor as the α_1 subunit.

By contrast, no endogenous protein reactive to α_2 -subunit-specific guinea pig polyclonal antibody GP5 (ref. 5) was detected, although a positive reaction occurred in mouse skeletal muscle (Fig. 2). It seems therefore that the α_2 -subunit, and probably the δ -subunit to which it is disulphide-bonded^{1,4}, are not present in the LCa. 11 cells; the presence of the β - or γ -subunit has not been tested for specifically. Subunits α_2 and δ are glycosylated and retained on wheat germ agglutinin, together with their associated α_1 - and β -components^{1,14}. We tested whether L cells might have a functional homologue to the skeletal muscle α_2 -subunit which would not cross react with the antibody, yet might interact with the α_1 -subunit to form a complex which would be retained by a wheat germ agglutinin column. However, the LCa. 11 α_1 -subunit, when solubilized under conditions that lead to retention of the pentameric skeletal muscle or cardiac DHPR (A. T. Leung and K.P.C., unpublished observations), failed to be retained by wheat germ agglutinin (data not shown).

The three LCa cell lines, but not recipient *tk*⁻ L cells or control transfected LEM5 cells, expressed Ca^{2+} currents and LCa. 11 cells produced the largest currents with the greatest consistency (Fig. 3). The only discernible currents in the *Ltk*⁻ and LEM5 cells have been inward and outward rectifying K^+

FIG. 1 Expression of DHP α_1 -mRNA and DHP-specific binding sites in L cells transfected with skeletal muscle DHP α_1 -cDNA. a Hybridization blot analysis for the presence of DHP α_1 -mRNA in poly(A⁺) mRNA isolated from rabbit skeletal muscle, from three cell lines stably transformed from *tk*⁻ to *tk*⁺ in the presence of pEV-DHP α_1 -DNA and from the recipient *Ltk*⁻ cell line. b Binding of the dihydropyridine Ca^{2+} -channel antagonist [³H]-(+)-PN200-110 to membranes from stably transformed cells expressing DHP α_1 -mRNA, but not to membranes from control recipient *Ltk*⁻ cells. The skeletal muscle α_1 -cDNA was cloned from a rabbit skeletal-muscle cDNA library constructed in the Okayama-Berg pCD vector²⁰ by MacLennan *et al.*²¹ and the complete open reading frame of the cDNA placed under the control of the mouse metallothionein-1 promoter in plasmid EV-142 (ref. 9) (gift from Dr R. Palmiter, University of Washington), to give pEV-DHP α_1 -tot (E. P.-R. and L.B., unpublished observations). This plasmid was transfected by the calcium phosphate method²² into murine thymidine kinase negative (*tk*⁻) L cells²³. Twenty individual cell clones were isolated with the aid of cloning rings after selection for *tk*⁺ cells using media and conditions described previously¹¹ and expanded in minimum essential medium alpha medium (GIBCO) in the presence of 10% fetal bovine serum, penicillin/streptomycin and hypoxanthine, aminopterin and thymidine. Before collecting or testing, cells were plated at the equivalent density of $\sim 2 \times 10^6$ cells per 100-mm dish and grown to confluence (3 days) to densities of $\sim 10 \times 10^6$ cells per 100-mm dish. After a medium change and addition of 100 μM ZnSO₄ for 15 h to induce maximal DHP α_1 -mRNA levels and its translation, the cells were either collected for analysis of mRNA, DHP binding to membranes or immunoblotting of membrane proteins, or used for electrophysiological analysis of Ca^{2+} channel activities.

METHODS. RNA analysis: Poly(A⁺) RNA was isolated from total RNA prepared by the guanidine-isothiocyanate/CsCl method using oligo (dT)-cellulose chromatography and electrophoresed through a 1.2% agarose gel in the presence of 50 mM sodium phosphate (P_i) buffer, pH 7.0, and 6.7% formaldehyde. The RNAs were transferred to Hybond-N membranes (Amersham). The membrane was then dried for 2 h at 80 °C, soaked in 6 × SSC, 5 × Denhardt's (1 × Denhardt's: 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone), 0.3 M P_i, pH 7.0, 0.5% SDS, 100 μg ml⁻¹ sheared herring sperm DNA and 20% formamide for 4 h at 37 °C, and hybridized overnight at the same temperature in the same medium with 10⁷ c.p.m. ml⁻¹ of nick-translated SacII(650)/SacI(5313) (ref. 3) fragment of the DHP α_1 -cDNA (specific activity 2.5 × 10⁸ c.p.m. μg^{-1}). The membrane was then washed at increasing stringency ending with 0.1 × SSC plus 0.5% SDS at 62 °C, sealed into a polyethylene bag and autoradi-



graphed for 30 h at -70 °C in the presence of two enhancing screens. For DHP-binding experiments, cells were induced with 100 μM ZnSO₄ for 15 h, rinsed twice with ice-cold 1 mM EDTA, 50 mM Tris-HCl, pH 7.2, scraped into ~4 ml of the rinsing medium with a rubber policeman, and homogenized in a Wheaton glass/teflon homogenizer (10 slow strokes at ~500 r.p.m.). The suspension was centrifuged at 420g for 5 min, the pellet was resuspended in 4 ml medium, rehomogenized and recentrifuged. The two low-speed supernatants were combined and centrifuged at 90,000g for 30 min. The pellet (membranes on figure) was resuspended in 50 mM Tris-HCl, pH 7.2, 1 mM EDTA. Typically, one 100 mm dish of confluent, Zn-treated cells yielded 500 μl of a membrane suspension with 1–1.5 mg protein ml⁻¹ containing 60–70% of the specific binding sites. Binding assays were in a final volume of 1 ml at room temperature for 90 min in the presence of 1 mM CaCl₂, 0.5 mM MgCl₂, 50 mM Tris-HCl, pH 7.2 and 0.4 nM [³H]-(+)-PN200-110 (8.0 Ci mmol⁻¹; Amersham). At the end of the incubation the samples were filtered under vacuum through Whatman GF/B glass fibre filters, washed 5 times with 5 ml of ice-cold 50 mM Tris-HCl, pH 7.2 with the aid of a Brandel filtration apparatus. Bound [³H](+)-PN200-110 was then determined by counting the radioactivity retained on the glass fibre filters using a liquid scintillation counter.

currents (data not shown). In the presence of Bay K 8644 (1–10 μM), 80% of LCa.11 cells with gigaohm input resistances gave rise to detectable Ca^{2+} tail currents. These currents were voltage-dependent, and were increased by the DHP agonist Bay K 8644 (ref. 15). The currents were blocked by Co^{2+} or Cd^{2+} , but tail currents of reduced amplitude persisted in the presence of Cd^{2+} (ref. 16). At voltages near threshold we occasionally observed single-channel events during the step and afterwards during the tail, and calculated from this a unitary conductance of 6 pS, which compares reasonably well with the majority value of 10 pS recorded under similar ionic conditions for Ca^{2+} channels of skeletal-muscle T-tubules incorporated into planar lipid bilayers¹⁷. From these tail-current amplitudes recorded with satisfactory voltage control, we estimated at least 400–800 chan-

nels per cell, which agrees with the estimated number of binding sites.

The Ca^{2+} currents rose very slowly (Fig. 3e). The fastest half-times (~ 500 ms) were considerably slower than the activation half-times (~ 50 ms) of Ca^{2+} currents in skeletal muscle¹⁸ and phenotypic cell lines of skeletal muscle¹⁹, which are themselves much slower than the onset of Ca^{2+} currents in neurons, cardiac and smooth muscle cells. Deactivation of the tail currents reflects the slowness of the activation process and inactivation (not shown) is slower still.

Our results show that the protein encoded by the α_1 -subunit cDNA is the DHP receptor and confirm the notion that this subunit is directly involved in Ca^{2+} permeation⁶. Moreover, it seems that the α_1 -subunit can function as a Ca^{2+} channel in

FIG. 2 Immunoblot analysis for presence of immunoreactive α_1 - and α_2 -proteins in membranes from LCa.11 cells and murine skeletal-muscle membranes, both positive for DHP binding, and in membranes from the recipient Ltk⁻ cells. Membranes from LCa.11 (600 μg per lane) and Ltk⁻ (600 μg per lane) cells were prepared as described above, except that the final resuspension was in a smaller volume to give concentrations of 15–30 mg ml^{-1} . Murine skeletal muscle membranes (50 μg per lane; 1.4 pmol DHP-binding sites per mg protein) were prepared as described in Knudson *et al.*²⁴, and pentameric rabbit skeletal muscle DHP-binding complex (2 μg per lane) was purified from triad membranes¹³. Membranes and purified protein were subjected to SDS-PAGE (3–12% gradient gels) using the Laemmli buffer system and then transferred to nitrocellulose according to Otter *et al.*²⁵. Nitrocellulose blots were blocked with 150 mM NaCl, 50 mM P_i , pH 7.4, containing 5% (w/v) nonfat dry milk, and then incubated with either monoclonal antibody IIF7 directed against the DHPR α_1 -protein (Anti- α_1) or affinity-purified polyclonal antibody GP5 directed against the α_2 protein (Anti- α_2). The specificities and properties of these antibodies have been described^{5,12}. The transfers were then incubated with horseradish peroxidase-conjugated secondary antibodies, and developed using 4-chloro-1-naphthol as substrate. Relative molecular mass standards ($\times 10^{-3}$) were prestained; calibrations are shown to the left and right.

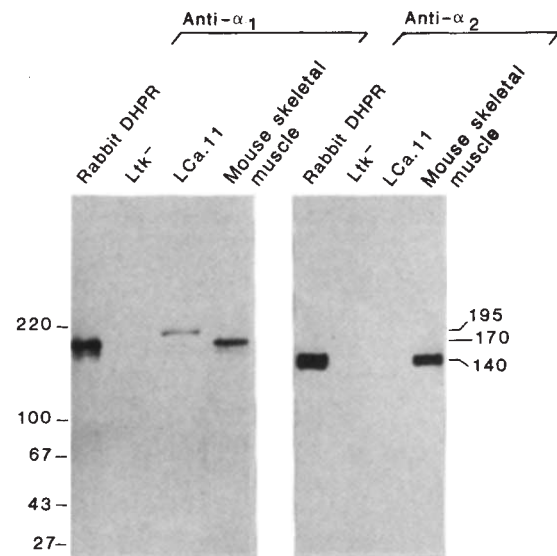
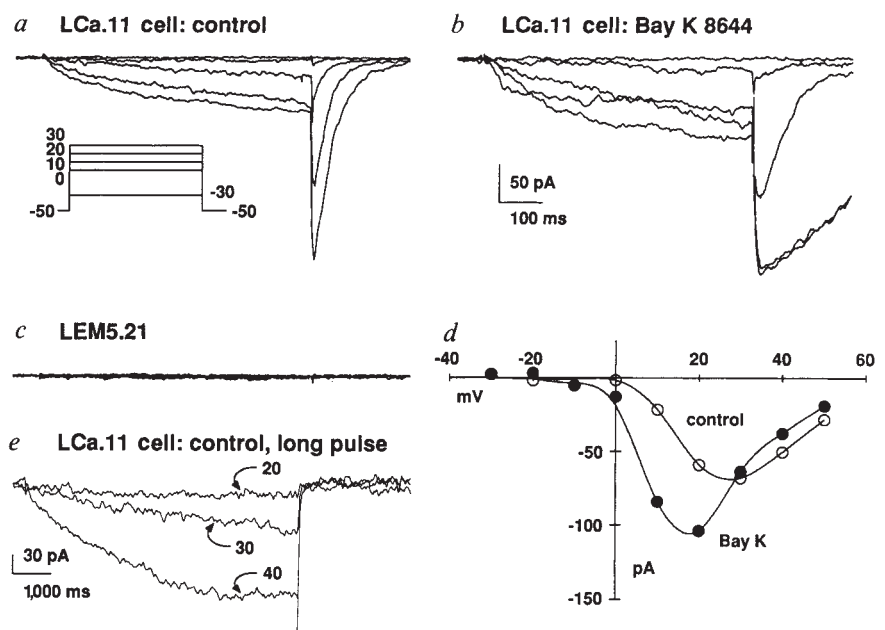


FIG. 3 Ca^{2+} -channel currents of an LCa.11 cell stably transformed with the DHPR α_1 -subunit cDNA before (a) and after (b) addition of Bay K 8644, and current-voltage relationships derived therefrom (d). Bay K 8644 at 2 μM produces typical effects: an increase in peak currents and a hyperpolarizing shift in activation at lower potentials. Voltage control was lost during the two largest tail currents shown in b, possibly because of blockage of the pipette. As control, LEM5 cells were stably transformed in exactly the same manner, except that the coding sequence was for the rat M5 muscarinic acetylcholine receptor (c).

METHODS. The gigaseal patch clamp method was used to measure currents²⁶. The second and third peak current-voltage relationships were obtained before (a) and after (b) the addition of (–) Bay K 8644 at 2 μM . The first and second I–V curves during control were the same. The cell was held at -50 mV and the 300-ms pulses shown in the insert of a were delivered at 0.1 Hz. The same protocol was used in c. In e, pulses were 7 s in duration and the currents displayed were produced by test pulses to 20, 30 and 40 mV delivered at intervals of one minute. Bath solution contained (in mM): 115 BaCl_2 , 10 HEPES (pH adjusted to 7.4 with n-methyl-D-glucamine (NMG)) and Ba^{2+} was the charge carrier through the Ca^{2+} channels in all experiments. Pipette solution was (in mM): 130 NMG, 15 EGTA, 5 BAPTA, 11.5 MgCl_2 , 8 CaCl_2 , 3 Na_2ATP , 0.1 Na_2GTP (pH adjusted to 7.2 with MES). Current records were corrected for linear capacitive and leak currents online by a P/4 procedure with four 0.25 amplitude-inverted steps from the holding potential of -50 mV and preceding the depolarizing step. Records in a, b, and c were filtered to -3 dB at 300 Hz and digitized at 600 Hz to 1.7 kHz; in e the corner



frequency f_c was 6 Hz and the sampling rate was 50 Hz. For the experiments using inorganic blockers, pulses were 630 ms in duration. Co^{2+} at 10 mM and Cd^{2+} at 5 mM were added as chloride salts to a static bath and blocked all components of Ba^{2+} current during the voltage step, revealing outward currents probably carried by Cs^+ through K^+ channels. Larger concentrations of these blockers were necessary in the presence of isotonic Ba^{2+} .

the absence of the α_2 - and probably δ -subunits; indeed the α_1 -subunit alone may suffice for these functions, although this has yet to be determined.

Perhaps as important are our findings that the transfected protein was significantly larger than rabbit skeletal-muscle protein and produced much slower Ca^{2+} currents. This could reflect an intrinsic deficiency in the processing capacity of the L cell. Another possibility, however, based on the finding that the α_1 -subunit is purified from its natural T-tubule environment along with the α_2 -, β -, γ - and δ -subunits, is that to acquire 'skeletal muscle' properties, α_1 must associate with one or more of these components which may be missing in LCa_v1.1 cells.

The present report demonstrates that stable expression of voltage-gated Ca^{2+} channels in tissue culture cells is possible. This adds stable transformation of cells, excitable or non-excitable, to the growing list of methods for studying the structure and function of voltage-gated ion channels. □

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GTP-activated communication between distinct inositol 1,4,5-trisphosphate-sensitive and -insensitive calcium pools

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INOSITOL 1,4,5-trisphosphate (InsP₃) is an established mediator of intracellular Ca^{2+} signals¹ but little is known of the nature and organization of Ca^{2+} regulatory organelles responsive to InsP₃. Here we derive new information from the study of Ca^{2+} movements induced both by InsP₃ and a specific GTP-activated Ca^{2+} translocation process^{2,3}. The latter mechanism is clearly distinct from that activated by InsP₃ (ref. 4) and may involve the translocation of Ca^{2+} between compartments without its release into the cytosol⁵⁻⁷. This idea is supported by the fact that GTP activates Ca^{2+} movement into the InsP₃-releasable pool^{7,8}. In the light of this evidence we postulated that there are two intracellular Ca^{2+} pools distinguishable by InsP₃-sensitivity and oxalate-permea-

bility, and that movement between them is activated by GTP⁷. We report here direct evidence for the existence and separation of two distinct Ca^{2+} -pumping compartments with properties coinciding with those predicted. Of these, the InsP₃-sensitive Ca^{2+} pool is identified within a purified rough endoplasmic reticulum fraction, an observation consistent with recent InsP₃ receptor-localization studies⁹. Ca^{2+} translocation between pools may reflect function of a class of small GTP-binding proteins known to mediate inter-organelle transfer in eukaryotic cells¹⁰.

In our previous studies we measured InsP₃- and GTP-mediated Ca^{2+} fluxes in both permeabilized cells^{3,7} and microsomal membrane preparations^{11,12}. Starting with endoplasmic reticulum (ER)-enriched microsomes from the DDT₁MF-2 smooth

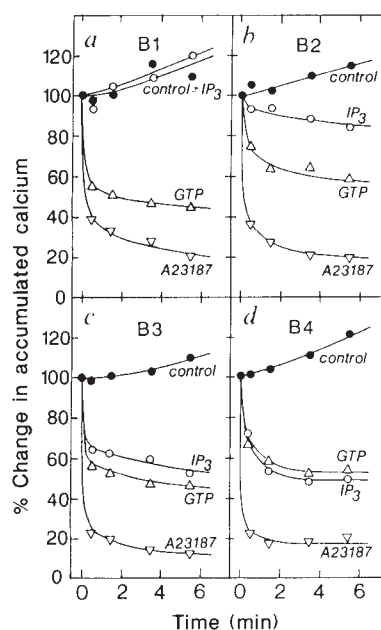


FIG. 1 InsP₃- and GTP-activated Ca^{2+} release from density-separated microsomal membrane vesicles isolated from DDT₁MF-2 cells. Membrane vesicles used in panels a, b, c and d were derived from the B1, B2, B3, and B4 fractions, respectively, as described below. Ca^{2+} uptake proceeded for 6 min before addition of either 10 μM InsP₃ (○), 10 μM GTP (△), 5 μM A23187 (▽), or control buffer (●).

METHODS. DDT₁MF-2 smooth muscle cells derived from hamster vas deferens were cultured as described⁵. Procedures for isolation and fractionation of microsomal membrane vesicles from cells have been described¹¹. ER-enriched microsomal membranes were separated by continuous sucrose-gradient centrifugation giving four consistently discrete bands of material with densities 1.11, 1.14, 1.17 and >1.18, representing 0.5, 0.8, 1.3 and 1.1% of the total cell protein, and designated B1, B2, B3, and B4, respectively¹¹. Relative to the cell homogenate, all fractions were enriched in glucose 6-phosphatase and NADPH-cytochrome c reductase activities (from 5- to 8-fold) and had high levels of $(\text{Ca}^{2+} + \text{Mg}^{2+})\text{ATPase}$ activity (up to 15-fold). The RNA contents of B1, B2, B3, and B4 were 71, 79, 302, and 389 $\mu\text{g mg}^{-1}$ protein, respectively. Electron microscopy revealed B3 and B4 fractions (which were very close in density) to comprise almost exclusively densely ribosome-studded RER vesicles. B4 also contained many free ribosomes. B1 and B2 fractions contained only smooth vesicular structures and no visible ribosomes. The fractions were almost devoid of mitochondrial marker enzyme activity; significant plasma membrane enzyme activity (5'-nucleotidase and ouabain-sensitive $(\text{Na}^{+} + \text{K}^{+})\text{ATPase}$) was observed only in the B1 fraction (up to 7-fold more than the homogenate). Accumulation of $^{45}\text{Ca}^{2+}$ was measured at 37 °C by rapid La^{3+} -quench and filtration procedures^{5,28}. Uptake medium comprised 140 mM KCl, 10 mM NaCl, 2.5 mM MgCl_2 , 10 mM HEPES-KOH, pH 7.0, 50 μM CaCl_2 (with 160 Ci mol^{-1} $^{45}\text{CaCl}_2$; buffered to 0.1 μM with EGTA²⁹), 1 mM ATP, 3% polyethylene glycol, 1 mM dithiothreitol. For all four membrane fractions, Ca^{2+} accumulation before additions (100%) was between 5 and 10 nmol Ca^{2+} per mg protein.

muscle cell line, simple and effective density separation of vesicles was accomplished by sucrose gradient centrifugation to give four discrete fractions, as described in Fig. 1 and ref. 11. All fractions were highly active for ER-marker enzymes. The RNA content of the two densest fractions (>0.3 mg RNA mg^{-1} protein) was comparable with that of highly purified tissue-derived rough endoplasmic reticulum (RER) fractions¹³, an evaluation which was confirmed by electron microscopy.

All four fractions displayed similar Ca^{2+} -pumping activity. Additionally, all fractions released $>50\%$ of accumulated Ca^{2+} with $10 \mu\text{M}$ GTP (Fig. 1). GTP-activated Ca^{2+} release from vesicles was exactly as described for permeabilized cells³⁻⁵, being rapid (maximal within 30 s), sensitive ($\text{EC}_{50} < 1 \mu\text{M}$ GTP), and specific for hydrolysable guanine nucleoside triphosphates. Only the densest fractions, however, responded to InsP_3 . Thus, $10 \mu\text{M}$ InsP_3 induced no Ca^{2+} release from the B1 fraction and had only a slight effect on B2 vesicles. Conversely, InsP_3 induced Ca^{2+} release from the B3 and B4 fractions to the same extent as GTP. As with permeabilized cells⁵, the actions of InsP_3 and GTP were not additive; no greater release was observed when both were added together (data not shown). The lack of an effect of InsP_3 on B1 was not due to InsP_3 metabolism as combination of B1 with denser vesicles still permitted equivalent release from the latter. InsP_3 -induced Ca^{2+} release closely paralleled ^3H - InsP_3 binding to the fractions. Specific InsP_3 -binding sites in the B1, B2, B3, and B4 membranes were 0.04, 0.07, 0.34 and $0.42 \text{ pmol mg}^{-1}$ protein, respectively, with K_d for InsP_3 of $\sim 0.1 \mu\text{M}$ (close to the K_m for Ca^{2+} release¹¹). These sites were highly sensitive to blockade by heparin (IC_{50} approximately $1 \mu\text{g ml}^{-1}$), a specific and potent antagonist of InsP_3 -induced Ca^{2+} release¹¹ and InsP_3 -binding to its purified receptor¹⁴.

InsP_3 -effectiveness also correlated closely with oxalate permeability, a distinguishing property of the InsP_3 -sensitive Ca^{2+} pool as revealed in permeabilized cells^{6,7}. Whereas oxalate

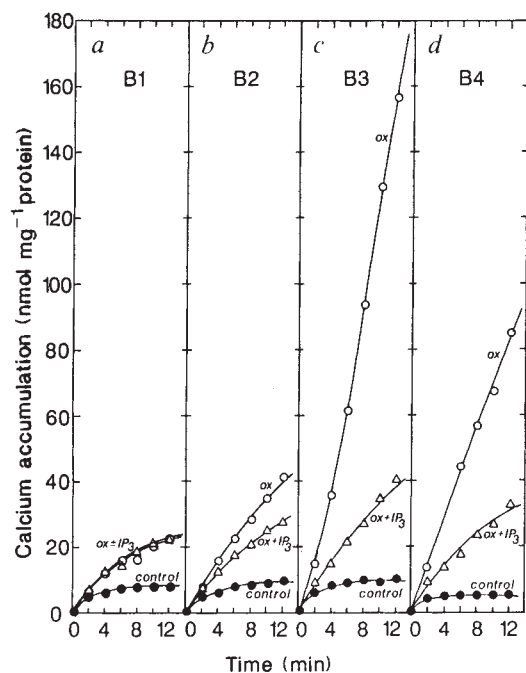


FIG. 2 Effect of oxalate on Ca^{2+} accumulation into B1, B2, B3, and B4 membrane vesicles (panels a, b, c and d, respectively) derived from DDT₁MF-2 smooth muscle cells. Ca^{2+} uptake commenced either with standard uptake medium as a control (●), or uptake medium containing 10 mM potassium oxalate with (△) or without (○) $10 \mu\text{M}$ InsP_3 , under the conditions described in Fig. 1.

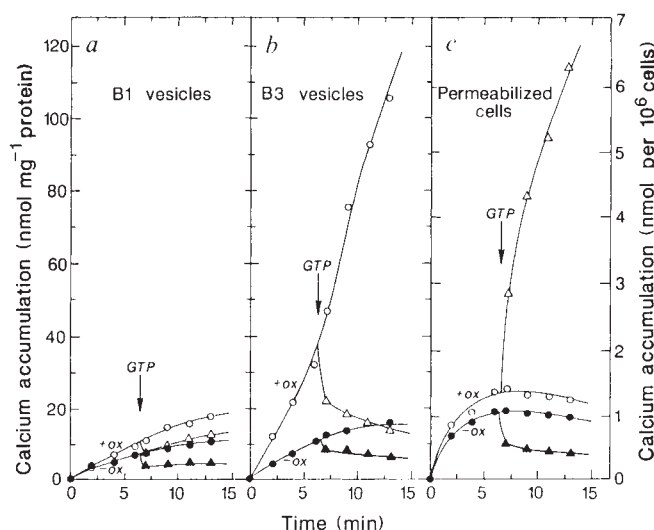


FIG. 3 Comparison of the effects of GTP in the presence and absence of oxalate on B1 vesicles, B3 vesicles, and permeabilized DDT₁MF-2 cells (panels a, b and c, respectively). Oxalate (ox) was either absent (closed symbols) or present (open symbols) at the start of uptake at 10 mM for vesicles (a and b) or 4 mM for cells (c). After 6.5 min, $10 \mu\text{M}$ GTP (▲, △) or control buffer (●, ○) was added. Note that with 10 mM oxalate, cells accumulate Ca^{2+} faster, and GTP still promotes a substantial increase in the rate of Ca^{2+} influx⁶.

effected only a modest enhancement of Ca^{2+} pumping into B1 vesicles, it induced a large enhancing effect (>15 -fold) on B3 and B4 vesicles (Fig. 2). In the latter fractions, InsP_3 prevented most of the oxalate-promoted Ca^{2+} accumulation, whereas InsP_3 induced little and no effects on the B2 and B1 fractions, respectively. Thus, excluding a small oxalate-promoted uptake component in all fractions, the denser fractions are clearly enriched in InsP_3 -sensitive, oxalate-permeable, Ca^{2+} -pumping vesicles. The combination of mediated oxalate entry and Ca^{2+} pumping result in Ca-oxalate precipitation and sustained Ca^{2+} accumulation. Efficient operation of InsP_3 -activated Ca^{2+} efflux from the same pool prevents this action. Permeability to oxalate is a property shared with muscle sarcoplasmic reticulum, probably reflecting the existence of a non-specific anion transporter¹⁵, the function of which may be to negate charge build-up during rapid Ca^{2+} release events⁶.

The action of GTP on vesicles differs in one important respect from its effects on Ca^{2+} movements within permeabilized cells. In the presence of oxalate, whereas GTP still induces Ca^{2+} release from vesicles, in permeabilized cells GTP induces marked uptake of Ca^{2+} (Fig. 3). With B1 vesicles, the effect of $10 \mu\text{M}$ GTP was independent of oxalate, with a similar release being observed in the absence and presence of oxalate, which is consistent with GTP activating Ca^{2+} release from the oxalate-impermeable compartment (Fig. 3a). The much larger oxalate-induced uptake into B3 vesicles was completely reversed by GTP, which is consistent with release from the oxalate-permeable pool (Fig. 3b). This contrasts markedly with the action of GTP on permeabilized cells, which, in the presence of oxalate, gives a dramatic uptake response to GTP (Fig. 3c). The difference in effect of GTP in the two systems is very important. We conclude that GTP-dependent uptake in permeabilized cells reflects GTP-activated intercompartmental translocation of Ca^{2+} , permitting access of Ca^{2+} accumulated in an oxalate-impermeable compartment to an oxalate-permeable compartment⁶. That only the release of Ca^{2+} can occur after homogenization indicates that, while GTP-activated translocation remains functional, communication between the separated pools is lost.

These studies address the key issues of identity and organization of intracellular Ca^{2+} pools. Regarding identity, the evidence that RER is the site of both binding and action of InsP_3 agrees well with immunocytochemical InsP_3 receptor-localization studies, which reveal heavy labelling of RER and nuclear envelope in neural cells⁹. Indeed, the two structures form a continuous single ribosome-bearing organelle¹⁶; the same Ca^{2+} -pump protein is expressed on both membranes¹⁷, and both structures accumulate large deposits of oxalate-precipitated Ca^{2+} (ref. 18). These properties of Ca^{2+} pumping and oxalate-permeability exactly coincide with those of the ribosome-rich membranes of the B3 and B4 fractions. Moreover, western analysis reveals specific localization within the B3 and B4 fractions (but not B1 and B2) of the slow cardiac Ca^{2+} pump and a protein recognized by calsequestrin antibodies (unpublished results). Such results further substantiate the presence of two distinct Ca^{2+} pools. The second (InsP_3 -insensitive) pool within the B1 and B2 fractions is not so well defined. These fractions are enriched in smooth ER; B1 also contains plasma membranes.

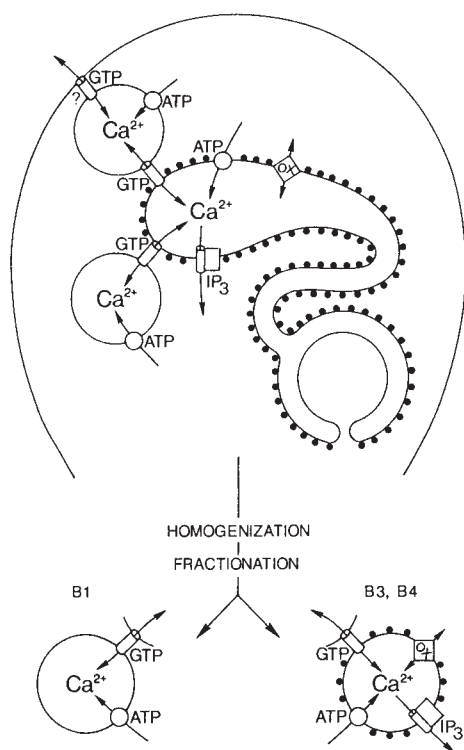


FIG. 4 Model depicting the InsP_3 -sensitive and -insensitive Ca^{2+} -pumping pools within cells, the putative GTP-activated translocation process between them, and the properties of the two types of vesicles derived from the pools by the fractionation procedure described in Fig. 1. GTP is depicted as activating Ca^{2+} transfer between the pools through junctional connections existing at close appositions between membrane surfaces⁵⁻⁷; connections are postulated to exist either between intact compartments or between intact compartments and non-enclosed membrane surfaces. Dense, ribosome-rich membrane vesicles present in the B3 and B4 fractions contain: (ATP+ Mg^{2+})-dependent Ca^{2+} -pumping activity, InsP_3 -activated Ca^{2+} release, InsP_3 -binding sites, mediated oxalate-entry (ox), and the GTP-activated Ca^{2+} transfer process (observed as Ca^{2+} release into the medium). These vesicles are considered to represent the continuous ribosome-rich compartment comprising the RER and nuclear envelope. The lighter, smooth membrane vesicles enriched in the B1 fraction (containing Ca^{2+} -pump and GTP-activated Ca^{2+} -release mechanisms) are of unknown cellular origin. This pool is depicted as communicating with the RER and/or the plasma membrane. Involvement of the plasma membrane is more speculative and is based on the observed plasma membrane content of the B1 fraction and on previous assertions^{6,7}.

The relationship between either of the two compartments and the 'calciosomes' (calsequestrin-containing, Ca^{2+} -pumping organelles, apparently distinct from but closely associated with ER) reported by Volpe *et al.*¹⁹ is unclear. This organelle, in contradistinction to ER, was proposed as the site of action of InsP_3 (ref. 21).

The data presented here, however, strongly suggest that RER itself is both a Ca^{2+} regulatory organelle and a major site of functional InsP_3 receptors—a conclusion reinforced by immunocytochemical studies⁹.

Regarding the organization of Ca^{2+} pools, a scheme based on our information and on evidence for GTP-activated Ca^{2+} translocation between pools through putative intracellular junctions^{6,7}, is given in Fig. 4. The opposite actions of GTP on permeabilized cells versus isolated vesicles (Fig. 3), suggest that, although the two pools and the connections between them are intact in cells, homogenization disrupts such connections. Dissociated vesicles still retain GTP-activated translocation but this results only in release to the medium rather than transfer between pools, indicating that the putative connections between the two intact compartments are very labile. Because the action of GTP seems to depend on close association between membrane surfaces⁵, it is possible that vesicles have not lost connections between membranes *per se*. Instead, the predominant connections may be between intact vesicles and non-intact membrane fragments (Fig. 4), explaining why it has not been possible to reconstitute Ca^{2+} transfer between isolated vesicle fractions by mixing.

What of the mechanism and significance of GTP-activated communication between Ca^{2+} pools? Studies strongly suggest that the process is related to the action of the group of small Ras-like GTP-binding proteins believed to mediate trafficking and inter-organelle transfer between ER, Golgi, and secretory vesicles by a process requiring GTP hydrolysis¹⁰. Recent analyses show that the vesicle fractions from DDT₁MF-2 cells contain a discrete pattern of at least seven distinct GTP-binding proteins (of relative molecular mass 21,000–28,000). After SDS-gel electrophoresis and transfer onto nitrocellulose, these proteins renature to reveal GTP-binding sites with nucleotide sensitivity and specificity (including differential blockade by GTP- γ S versus GppNHP^{3,6}) very close to GTP-activated Ca^{2+} translocation. Renaturation of GTP-binding on blots is a specific property of the small GTP-binding proteins (for example, Ras p21 (ref. 20), Ypt1p (ref. 21), and Sec4p (ref. 22)), as distinct from other GTP-binding proteins such as tubulin or G proteins. Recent evidence implicates the *ypt1* gene product in controlling an intracellular Ca^{2+} regulatory organelle in yeast²³. The mammalian equivalent of this protein may mediate transfer between ER and Golgi, a process clearly dependent on GTP-hydrolysis and regulated by Ca^{2+} (ref. 24). Significantly, western analysis reveals the presence of the mammalian Ypt protein specifically within those vesicle fractions showing Ca^{2+} -translocation responses to GTP isolated from DDT₁MF-2 and other cells (unpublished observations). Although the mechanism of these small GTP-binding proteins has not been elucidated, their effects¹⁰ are highly consistent with the action, nucleotide specificity, and GTP-hydrolytic requirement of the GTP-mediated Ca^{2+} -translocation process described here. The significance of GTP-activated transfer of Ca^{2+} may be to control the extent of the InsP_3 -releasable Ca^{2+} pool by recruiting closely associated InsP_3 -insensitive Ca^{2+} pools; indeed, RER cisternae heavily stained for InsP_3 receptors lie immediately adjacent to unstained cisternae⁹. Additionally, the GTP-activated Ca^{2+} transfer process could mediate vesiculated transfer of Ca^{2+} from outside the cell to replenish the InsP_3 -sensitive Ca^{2+} pool. The GTP effect, rather than being constitutively active, could be controlled by another intracellular effector, perhaps inositol 1,3,4,5-tetrakisphosphate, which, like GTP, has been shown to mediate Ca^{2+} entry into the InsP_3 -releasable pool^{25,26}. Lastly, the existence and coordinated emptying of InsP_3 -sensitive and -insensitive

Ca^{2+} pools may have significance in the generation of effector-evoked Ca^{2+} oscillations²⁷. □

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Resident pulmonary lymphocytes expressing the γ/δ T-cell receptor

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THE biological role of cells bearing the γ/δ T-cell antigen receptor (TCR) is as yet unclear^{1–3}. Although there are indications that some γ/δ^+ cells can mediate cytotoxicity^{4–7}, their antigen-related functions have not yet been defined. In the mouse, γ/δ^+ cells constitute 1–3% of T cells in lymphoid organs^{8,9}. Intestinal intraepithelial lymphocytes (IELs) and dendritic epidermal cells (DECs) also appear to carry the γ/δ TCR^{6,10–13}. The strategic locations of DECs and IELs have led to the suggestion that γ/δ^+ cells could constitute a first line of defence in the vicinity of large surfaces of contact with the environment¹⁴. We report here that an estimated 8–20% of resident pulmonary lymphocytes (RPLs) are $\text{CD}3^+ \alpha\beta \text{ TCR}^-$, and presumably $\gamma/\delta \text{ TCR}^+$. Furthermore, mice exposed to aerosols containing a *Mycobacterium tuberculosis* extract have an increased number of activated $\text{CD}3^+ \alpha\beta \text{ TCR}^-$ pulmonary T cells which can be propagated *in vitro*.

A heterogeneous non-adherent cellular suspension (65% $\text{Thy}1^+$) was prepared from dissected lung fragments of BALB/c mice. Lungs were removed only after depletion of circulating blood by extensive perfusion through the pulmonary artery. The suspended cells were radioiodinated, lysed and subjected to

immunoprecipitation. Figures 1a and b show that the signal from γ/δ TCR molecules in the lysate of 2×10^6 cells is comparable with that from $\alpha\beta$ -TCR protein precipitated from the same cells. In contrast, only trace amounts of γ/δ TCR can be precipitated from the lysate of 2×10^7 splenic or lymph-node lymphocytes. Thus, among cells extracted from murine lungs, the γ/δ TCR molecule is expressed at a higher level than in lymphoid organs.

Immunofluorescence and immunoprecipitation indicate that most of the resident pulmonary lymphocytes (RPL) expressing the γ/δ TCR are $\text{CD}4^- \text{CD}8^-$ 'double negative'. Two-colour immunofluorescence analysis using anti-CD4, anti-CD8 and a pan-reactive anti- $\alpha\beta$ TCR monoclonal antibody (mAb)¹⁵ showed that the non-adherent cell suspension contained ~10% $\text{CD}8^+$ and 22% $\text{CD}4^+$ cells as two non-overlapping populations (Fig. 2a). Furthermore, virtually all $\text{CD}4^+$ and $\text{CD}8^+$ cells were $\alpha\beta \text{ TCR}^+$ (Fig. 2b). These measurements predict that lymphocytes expressing the γ/δ TCR belong to the double-negative pool. In support of this, the elimination of either $\text{CD}4^+$ or $\text{CD}8^+$ cells, or both, results in a significant increase in the amount of γ/δ protein precipitated from the same number of cells (Fig. 2c

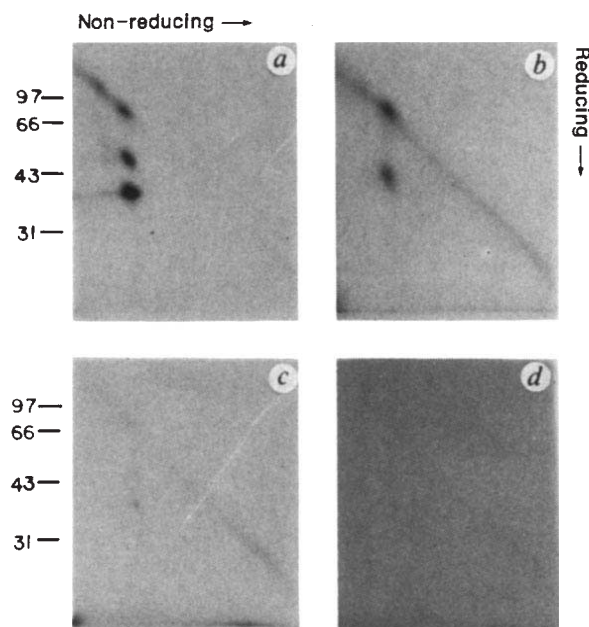


FIG. 1 Immunoprecipitation of γ/δ TCRs from non-adherent cells of the lung. Triton X100 lysate of 2×10^6 radiolabeled pulmonary lymphocytes were immunoprecipitated with an anti- γ antiserum (a) or anti- $\alpha\beta$ antiserum (b). Lysate from 2×10^7 lymph-node cells (c) or 2×10^7 splenocytes (d) were immunoprecipitated with the anti- γ antiserum.

METHODS. Lung perfusion was performed on 6–8-week-old BALB/c mice of either sex as described in ref. 20. Dissected lungs were cut into thin strips and stirred with PBS-EDTA (1 mM) for 40 min at 4 °C. Floating tissues were discarded and the cells collected by centrifugation. Viable cells were fractionated and collected from a Ficoll isopaque gradient (Pharmacia) and incubated in tissue culture flasks in IMDM medium +10% fetal calf serum (FCS) at 2×10^5 per ml, 37 °C, 5% CO_2 for 3 hours to remove adherent cells. Typical recovery is $\sim 5 \times 10^5$ cells per animal. The non-adherent cells were removed, surface-labelled with Iodogen (Pierce Biochemicals), and solubilized in 1% Triton X100, 10 mM iodoacetamide, 0.15 M NaCl, 1 mM phenylmethylsulphonyl fluoride, $1 \mu\text{g ml}^{-1}$ leupeptin and aprotinin, and 1 mM EDTA. The lysate was precleared with normal rabbit serum and protein A sepharose beads (Sigma), and incubated with either 10 μg biotin-conjugated affinity-purified rabbit anti- γ peptide antibody (C γ 1: CGNEKKS peptide) followed by streptavidin sepharose beads (BRL) or 10 μl rabbit anti- $\alpha\beta$ antiserum²¹ followed by protein A Sepharose beads. The immunoprecipitates were washed four times (1 h each) in the lysis buffer, and analysed on 10% SDS-PAGE under nonreducing (first dimension) and reducing (second dimension) conditions. Lymph-node cells and splenocytes were prepared by standard procedures, radiolabelled and immune precipitated in the same way.

and *d*). Taken together, these data indicate that most RPLs expressing the $\gamma\delta$ TCR are $CD4^-8^-$. In the absence of a panreactive anti- $\gamma\delta$ TCR-staining reagent, the frequency of $\gamma\delta^+$ cells in the non-adherent lung cell suspension was estimated as the fraction of cells that were $CD3^+$ but $\alpha\beta$ TCR $^-$. As shown in Fig. 3a, 19% of the cells seem to be $CD3^+ \alpha\beta$ TCR $^-$. But, many of the fresh RPLs express low levels of CD3 and are clearly $\alpha\beta$ TCR $^-$. It would be possible to divide the $CD3^+ \alpha\beta$ TCR $^-$ cells into two subsets: CD3 low (left of the arrow) and CD3 high (right of the arrow). In this case, the CD3 high $\alpha\beta$ TCR $^-$ cells represent 8% of the total cell suspension.

A surprising observation is the fact that $\alpha\beta$ TCR $^+$ 'double-negative' T cells are quite numerous among pulmonary lymphocytes (10% of the total fresh suspension of non-adherent cells) (see Fig. 2b). Although it has recently been reported that some $CD4^-8^-$ murine T cells can express the $\alpha\beta$ TCR, these cells constitute only a very minor fraction (0.5%) of murine thymocytes¹⁶. In contrast, the fraction of $\alpha\beta$ TCR $^+$ double-

negative lymphocytes in the lung represent at least 20% of the total $\alpha\beta$ TCR $^+$ cells.

If $\gamma\delta$ T cells were to play a part in immune defence, it should be possible to activate them by administration of appropriate antigens *in vivo*. Because RPLs are localized in the lungs, we attempted to activate them using aerosols generated from an isotonic solution of tuberculin-PPD (a mixture of *M. tuberculosis* antigens). The tropism of *M. tuberculosis* for the lung, and the good T-cell response elicited by many mycobacterial antigens, suggested to us that PPD would be a good candidate antigen for these studies. *M. tuberculosis*, which is used for the commercial preparation of PPD, is a potent inducer of experimental murine lung tuberculosis¹⁷. Moreover, this bacterium has been classified as being 'antigenically indistinguishable' from its natural murine counterpart *M. microti*¹⁸. We assumed that the local stimulation with PPD *in vivo*, if effective, would result in the specific triggering of a fraction of pulmonary lymphocytes. Besides their antigen-specific TCRs, such cells should carry lymphokine receptors. Thus, they should be directly and preferentially expanded *in vitro* in a culture medium containing either lymphokines or the priming antigen.

In one case, we compared a day-8 T-cell line obtained from pulmonary lymphocytes isolated from PPD-primed mice and expanded *in vitro* directly in the presence of interleukins (Fig. 3b), with a line obtained from pulmonary lymphocytes of mice exposed to ovalbumin (OVA) (Fig. 3c). A striking difference appeared in the recovery of $CD3^+ \alpha\beta$ -TCR $^-$ proliferating T cells: in the lines from PPD-primed mice, >85% of the growing cells were detected by anti-CD3 antibodies, of which about half were $\alpha\beta$ TCR $^-$, whereas the rest were $\alpha\beta$ TCR $^+$ (Fig. 3b). In contrast, in the line from mice primed with OVA, only a minority of the proliferating T cells were $CD3^+ \alpha\beta$ TCR $^-$ (Fig. 3c). Although a small fraction of RPLs isolated from unimmunized mice could be expanded *in vitro* if lymphokines were present at the start of culture, over 80% of the cells enriched under such condition were $\alpha\beta$ TCR $^-$, whereas ~10% were $CD3^+ \alpha\beta$ TCR $^-$ (Fig. 3f). These data indicate that exposure to PPD *in vivo* is required for the further enrichment of $CD3^+ \alpha\beta$ -TCR $^-$ pulmonary lymphocytes *in vitro*.

In an alternative experiment, pulmonary lymphocytes isolated from mice exposed to either PPD or OVA were expanded for 4 days *in vitro* in the presence of PPD or OVA respectively. Their proliferation was then continued in medium enriched with lymphokines until day 7, when the proportion of $CD3^+ \alpha\beta$ -TCR $^+$ and $CD3^+ \alpha\beta$ -TCR $^-$ cells was estimated by fluorescence analysis. Under these conditions, there was a significant enrichment of $CD3^+ \alpha\beta$ -TCR $^-$ lymphocytes by PPD (Fig. 3d), but not with OVA (Fig. 3e). Thus, in the absence of lymphokines during the first 4 days, the presence of the priming antigen in culture was essential for the survival of both $CD3^+ \alpha\beta$ TCR $^+$ and $CD3^+ \alpha\beta$ -TCR $^-$ cells. Indeed, cell recovery from control cultures which did not contain the priming antigen was low, and among the few $CD3^+$ cells collected at day 7, $\alpha\beta$ TCR $^-$ lymphocytes represented a negligible fraction. Results were similar from cultures of unprimed cells presented for the first 4 days *in vitro* with PPD or OVA in the absence of lymphokines. Taken together, these experiments show that *M. tuberculosis* extracts contain at least one active substance responsible for events which lead to a selective triggering and proliferation of $CD3^+ \alpha\beta$ TCR $^-$ RPLs *in vivo* and *in vitro*.

It has been reported^{10,13} that $\gamma\delta$ T cells located in different tissues selectively expressed different V γ gene segments. Thus, clones of murine DEC γ s display predominantly V γ 5 and V δ 1 (ref. 13), whereas IELs preferentially use V γ 7 (ref. 10). Our preliminary data suggest that in RPLs use of $\gamma\delta$ V gene segments is not random, and that V γ 4, V δ 1 and V δ 6 are selectively expressed. This indicates that lung-specific lymphocytes use well defined V γ and V δ segments which distinguish them from other $\gamma\delta$ cells present in either lymphoid organs or other epithelial tissues.

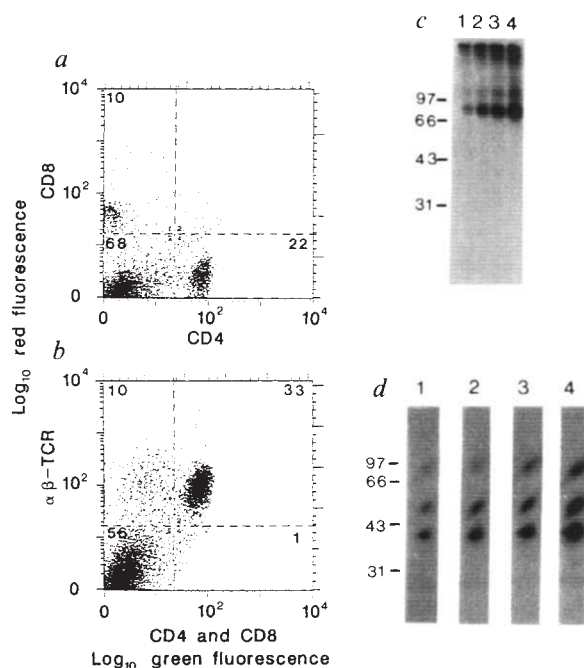
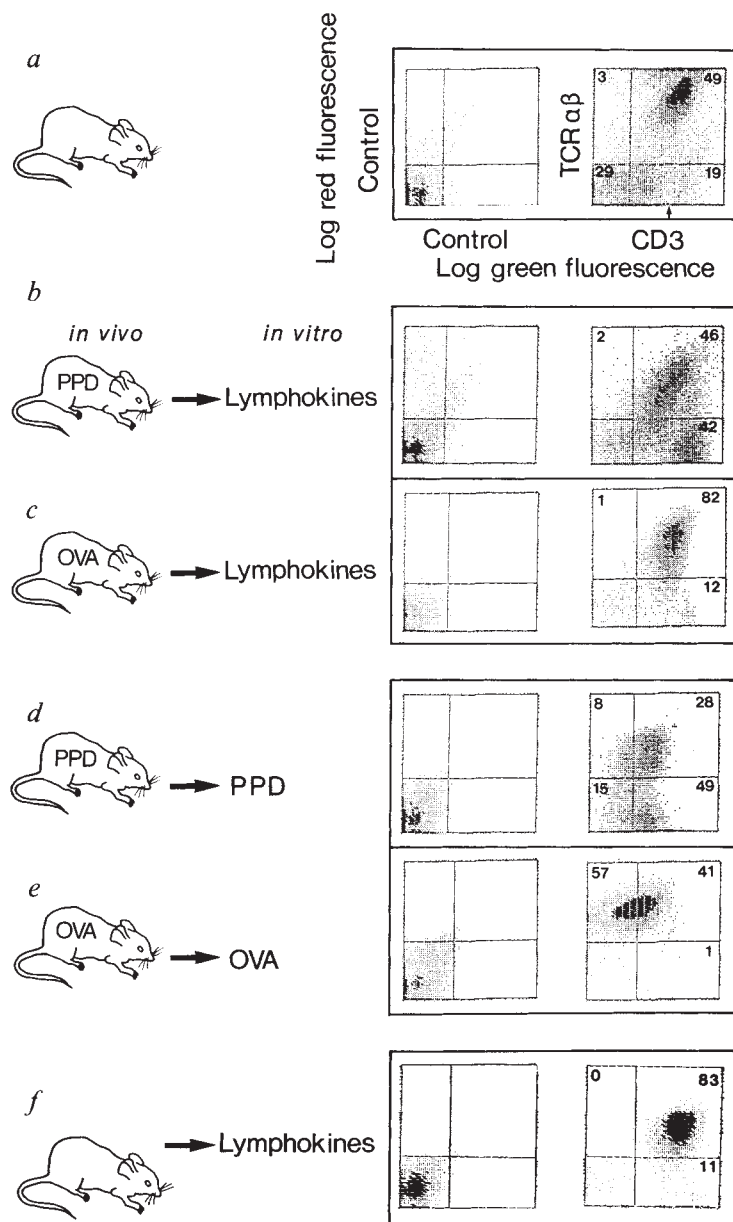


FIG. 2 Phenotypic characterization of non-adherent resident pulmonary lymphoid cells. Cells (5×10^5) were stained with *a*, biotin-conjugated anti-CD8 (mAb 53.6) followed by streptavidin phycoerythrin (TAGO), and fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (mAb GK1.5) or *b*, biotin conjugated anti- $\alpha\beta$ -TCR (mAb H57-597) followed by streptavidin phycoerythrin and FITC-conjugated anti-CD8 (mAb 53.6) and anti-CD4 (mAb GK1.5). Two-colour flow cytometry analysis was performed on a Becton Dickinson FACScan and the data for 2×10^4 cells were calculated with the FACScan software. In *c* and *d*, lanes 1, 2, 3 and 4 are $\gamma\delta$ immunoprecipitation of RPLs, prepared and immunoprecipitated with an anti- $\gamma\delta$ anti-serum as in Fig. 1 with the following modifications: cells were either untreated (lane 1) or treated with anti-CD8 (lane 2), anti-CD4 (lane 3), or both anti-CD4 and anti-CD8 (lane 4), followed by Mar 18 and rabbit complement to eliminate the appropriate antigen-bearing cells. Cells were then cultured for 7 days in IMDM plus 10% FCS in the presence of 15 U ml^{-1} rIL-1 (gift from P. Lomedico), 10 U ml^{-1} rIL-2 analogue (AMGEN), 30% WEHI-3-conditioned supernatant, 10 ng ml^{-1} TPA (12-O-tetradecanoyl phorbol 13-acetate) and $1 \text{ } \mu\text{M}$ ionomycin for the first 48 hours, and subsequently in medium without TPA and ionomycin. After 48 h, cells were collected and reset in culture at a cell density of 2×10^5 per ml for the first 24 h and at 10^5 per ml for the next 24 h, and seeded in fresh medium every second day at a concentration of 5×10^4 per ml. In these short-termed cultures, the depletion of CD4- and/or CD8-bearing cells was confirmed by flow cytometry. Each lane represents 5×10^5 cell lysate equivalent analysed under non-reducing conditions (*c*), and two-dimensional analysis (*d*) as in Fig. 1. Only the appropriate regions of the gels are compared in *d*.

FIG. 3 Activation of $CD3^+ \gamma\delta$ -TCR $^-$ resident pulmonary lymphocytes by PPD. Cells were prepared for two-colour flow cytometry essentially as in Fig. 2, using the same anti- $\alpha\beta$ -TCR on the y axis, and FITC-conjugated anti-CD3 (145-2C11) on the x axis in a-f on the right panels. An Epics C flow cytometer was used in these analyses. a, Freshly isolated RPLs; b, RPLs from mice immunized with PPD and cultured for 7 days in lymphokines; c, same as b, except that mice were immunized with OVA; d, RPLs from mice immunized with PPD, cultured for 4 days in PPD without lymphokines, and then for three more days in PPD plus lymphokines; e, same as d except that OVA was used instead of PPD; f, RPLs from unprimed mice, cultured for 7 days in lymphokines.

METHODS. Mice were exposed daily for a period of 5 minutes to aerosols generated from a solution containing either soluble PPD ($0.5 \mu\text{g ml}^{-1}$) or ovalbumin (OVA, $50 \mu\text{g ml}^{-1}$). These doses were determined empirically. Mice were placed in a small cylindrical chamber, connected to a DeVilbiss Nebulizer (Model 644) and the chamber saturated with aerosols generated by the nebulizer according to the manufacturer's specifications. PPD was obtained from Connaught Lab or from Dr M. Magnuson at the Staten Serum Institute, and OVA from Sigma. After 7 days of daily aerosol immunizations, mice were rested for another 7 days and finally were re-exposed for four more days to the same antigens. Twenty-four hours after the last immunization, pulmonary lymphocytes were isolated, as for control mice in Fig. 1. In mice exposed to tuberculin, the total yield of pulmonary lymphocytes was 10^6 to 1.5×10^6 cells per mouse ($2-3 \times$ higher than the yield obtained from nonimmunized mice or OVA-immunized mice). Cells were cultured in medium supplemented with lymphokines as described for Fig. 2, except that no TPA or ionomycin was used. In d and e, cells were cultured for the first 4 days in IMDM plus 10% FCS in the presence of either $5 \mu\text{g ml}^{-1}$ PPD or $50 \mu\text{g ml}^{-1}$ OVA (determined empirically) at a cell density of 10^6 per ml, and subsequently expanded as for c and d.



The presence of $\gamma\delta$ lymphocytes in the lung strengthens the idea that some $\gamma\delta$ cells home to specific anatomical sites (preferentially epithelia) which are in contact with the environment. The distinct usage of the V gene, specific for each tissue, could either reflect the reactivity of such cells to tissue-specific pathogens or their recognition of tissue-specific restriction elements, similar to those of the major histocompatibility complex (MHC). In either case, the specific triggering of pulmonary $\gamma\delta$ lymphocytes in murine lungs exposed to PPD is compatible with the existence of a local mechanism of defence against lung tuberculosis which involves $\gamma\delta$ T cells. It is possible that PPD preparations contain ligands for the $\gamma\delta$ TCR of some pulmonary lymphocytes. We will not speculate whether such ligands could be exclusively $\gamma\delta$ -T-cell immunogens or whether they could also induce an $\alpha\beta$ -T-cell response. We are trying to identify and purify such ligands in the hope of determining whether the triggering of $\gamma\delta$ pulmonary T cells requires processing-dependent antigen presentation and restriction to a specific polymorphic MHC-like molecule, or whether $\gamma\delta$ TCRs transduce inductive signals through direct binding of such ligands, aggregated or in solution. □

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Repression of transcription mediated at a thyroid hormone response element by the *v-erb-A* oncogene product

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SEVERAL recent observations^{1,2}, such as the identification of the cellular homologue of the *v-erb-A* oncogene as a thyroid-hormone receptor^{3,4}, have strongly implicated nuclear oncogenes in transcriptional control mechanisms. The *v-erb-A* oncogene blocks the differentiation of erythroid cells, and changes the growth requirements of fibroblasts and erythroblasts⁵⁻⁷. Mutations in *v-erb-A* protein have led to the loss of its affinity for thyroid hormones^{3,8} but do not affect its DNA-binding ability^{8,9}, a property required for biological activity⁹. We report here the identification of a novel thyroid-hormone response element (TRE) in the long terminal repeat of Moloney murine leukaemia virus that binds the *c-erb-A-α* protein. The *v-erb-A* protein abolishes the responsiveness of this TRE to thyroid hormone, although it has a lower affinity than the normal receptor for the TRE. The data indicate that overexpressed *v-erb-A* protein negatively interferes with normal transcriptional-control mechanisms, and that amino-acid substitutions have altered its DNA-binding properties.

Studies addressing the effects of *c-erb-A* expression from a Moloney murine leukaemia virus (MoMLV)-based retroviral vector¹⁰ revealed increased expression of the integrated provirus in response to thyroid hormone (A.M. *et al.*, manuscript submitted). The induction occurred at the transcriptional level, and was insensitive to cycloheximide, thus suggesting a direct interaction between a TRE and the receptor. To confirm that viral regulatory sequences were directly responsible for this effect, we used a reporter plasmid (pMLV; ref. 11) containing MoMLV LTR sequences followed by the herpes simplex virus (HSV) thymidine kinase (*tk*) promoter. The MoMLV long terminal repeat (LTR) promoter (including TATA box and CAP site) is absent in this construct, and transcription has been reported to start correctly from the *tk* promoter, driving expression of the gene for chloramphenicol acetyltransferase (CAT)¹¹. In transient-expression assays (Fig. 1), thyroid hormone treatment led to a clear increase of CAT activity (lanes 1 and 2), an effect which could be enhanced further by co-transfection of a *c-erb-A*-expression plasmid pKCR2-CEA (lanes 3 and 4). A deletion mutant (pΔMLV-CAT) containing only the 75-base-pair (bp) repeats of the LTR (which constitute the enhancer function of the LTR¹²), or a reporter plasmid containing the CAT gene under the control of the Rous sarcoma virus (RSV) LTR (data not shown) were not hormone responsive, demonstrating that increased CAT expression was not due to a general increase of transcription in response to thyroid hormone.

We confirmed the possibility of a direct interaction between *c-erb-A* and regulatory sequences in the LTR using recombinant vaccinia viruses to obtain highly enriched preparations of *c-erb-A*. Nuclear extracts from vaccinia-vector infected cells contain ~2% *c-erb-A* which binds T₃ as well as DNA-cellulose with

the expected affinity, and can be used to study sequence-specific DNA binding by *c-erb-A* (J.S. *et al.*, manuscript submitted). The presence of a high-affinity binding site for *c-erb-A* in the MoMLV LTR was suggested by co-immunoprecipitation (data not shown) and gel-retardation experiments (see below). DNase I footprinting analysis revealed protection of a discrete sequence in the LTR by extracts containing *c-erb-A* (Fig. 2a), but not by control vaccinia extracts prepared in a similar manner from cells infected with wild-type vaccinia. The footprinted region (CAAGGACCTGAAATGACCCTG, nucleotides 331-351 in ref. 13) is located in the area of the LTR conferring T₃ induction, as shown above with the MLV constructs. The element is located downstream from the enhancer 75-bp repeats, ending ~15 nucleotides upstream of the CCAAT box (Fig. 1b). To confirm further the identity of this sequence as a TRE, an oligonucleotide (SAL, 21 nucleotides) corresponding to the protected sequence was introduced in both orientations into a reporter plasmid (pBLCAT2, ref. 14) upstream of the heterologous HSV-*tk* promoter. The resulting constructs (pSAL1-CAT and pSAL2-CAT, respectively) were responsive to thyroid hormone in a *c-erb-A*-dependent manner (Fig. 2b). Inspection of the TRE sequence revealed an imperfect palindrome, with partial homology (the TGACC motif) to oestrogen-responsive elements and a thyroid-hormone-responsive element in the promoter of the rat growth-hormone gene¹⁵. The presence of a 'gap' at the centre of dyad symmetry has been proposed to distinguish functional TREs from oestrogen-responsive elements¹⁶. But the gap between the TGACC homology and its partially palindromic counterpart GGACC in the MoMLV LTR would be five nucleotides long,

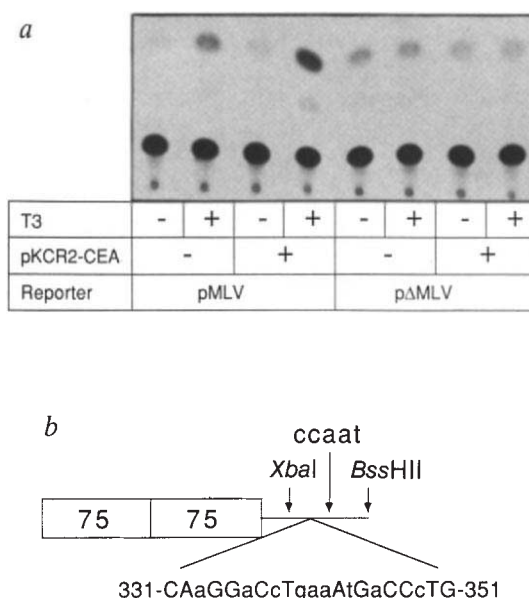


FIG. 1 a, Thyroid-hormone responsiveness of the MoMLV LTR. Reporter plasmids (5 µg) pMLV (ref. 11) or pΔMLV (lacking the sequence between the *Xba*I and *Bss*HII sites; see Fig. 1b) were used to transfect CV-1 cells in the presence of 0.2 µg pKCR2-CEA (a pKCR2-derived expression vector²³, containing the sequence encoding *c-erb-A-α* (ref. 3) under the control of the SV40 early promoter) or the same amount of pKCR2 vector as a control, using the Ca₃(PO₄)₂ co-precipitation method. Cells were treated or not with 25 nM T₃ in medium containing serum depleted of thyroid hormone, and CAT activity determined after 18 h. All CAT assays are normalized to an internal control (β-galactosidase activity from a co-transfected RSV-βgal plasmid), and a representative of several experiments is shown. b, Schematic representation of the MoMLV LTR, and location of the *c-erb-A*-binding site. The sequence of the SAL-oligonucleotide that functions as a TRE (nucleotides 331-351 from the beginning of the U3 region)¹³ is shown, with capitals illustrating its partially palindromic character.

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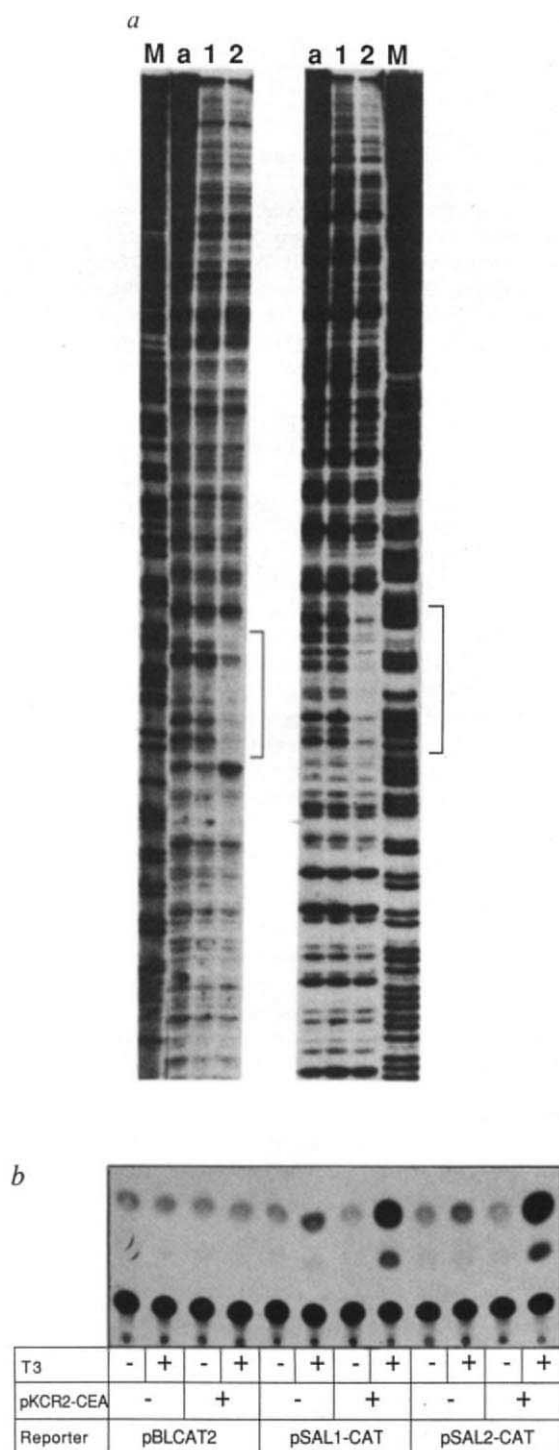


FIG. 2 *a*, DNase I protection by c-erb-A. An *EcoRI-NdeI* fragment (~ 2 fmol) from pMLV containing the entire LTR, and end-labelled at the *EcoRI* site (specific activity 2×10^4 c.p.m. per ng) was incubated with $10 \mu\text{g}$ nuclear extract at 30°C for 30 min in the presence of 0.1 mg ml^{-1} polyd(I-C), 40 mM KCl, 10 mM HEPES pH 7.8, 2 mM MgCl_2 , 1 mM DTT, 5% glycerol, and 0.1 mg ml^{-1} BSA, followed by limited DNase I digestion. Nuclear extracts were prepared from HeLa cells infected with either wild-type (control, lanes labelled 1) or recombinant chicken c-erb-A- α (ref. 3) expressing vaccinia virus (lanes labelled 2). Lanes labelled a: no extract added; lanes labelled M: Maxam-Gilbert G+A reaction. Extracts containing c-erb-A $\sim 2\%$ pure (manuscript in preparation). *b*, Ability of the protected sequence to function as a TRE when linked to a heterologous promoter. The SAL oligonucleotide (see Fig. 1*b*) was cloned into the *XbaI* site of pBLCAT2 (ref. 14) in both orientations, yielding pSAL1-CAT and pSAL2-CAT. Transfection and CAT assays were as in Fig. 1.

whereas our data show that such an element can still activate transcription in response to thyroid hormone.

Several questions regarding the mechanism of action of v-erb-A remain unanswered: first whether v-erb-A, which has lost its hormone-binding capacity, behaves as a constitutive repressor and/or an inducer of transcription, particularly given the fact that mutant steroid receptors can have such properties^{17,18}; second, whether the response elements recognized by v-erb-A are identical to those binding the normal thyroid-hormone receptor, as the viral protein has two amino-acid substitutions in its DNA-binding domain. We addressed these questions using the TRE in the MoMLV LTR as a model system. Transient-expression experiments with the TRE in the pMLV reporter plasmid showed that increasing doses of a co-transfected v-erb-A-expression plasmid (pKCR2-VEA) led to a gradual inhibition of the T_3 response mediated by the endogenous T_3 receptor in the transfected cells (Fig. 3*a*). In control experiments, v-erb-A affected neither the low expression of the pMLV and p Δ MLV constructs when assayed in the absence of T_3 , nor of p Δ MLV in the presence of T_3 . But, hormone responsiveness in the presence of v-erb-A could be

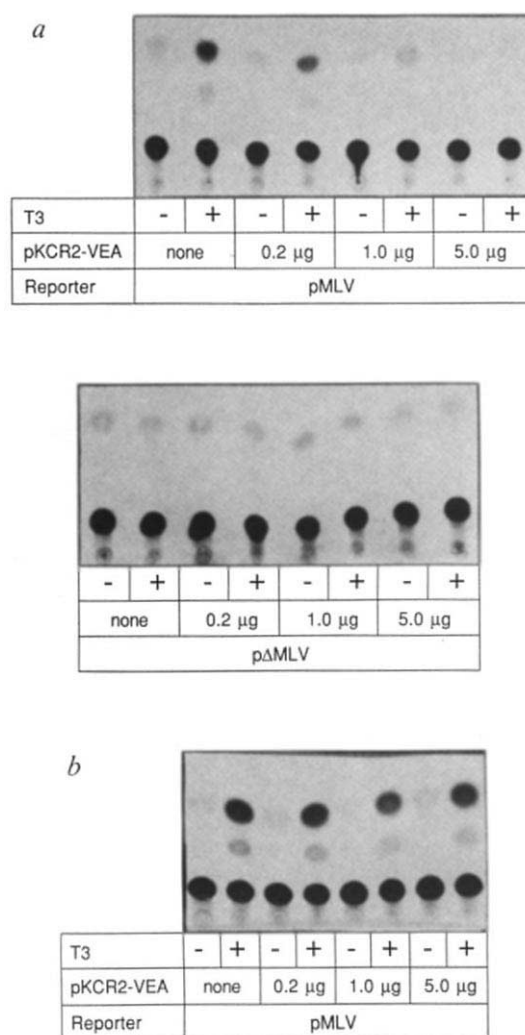


FIG. 3 Repression of TRE activity by the v-erb-A oncogene. The indicated reporter plasmids (5 μg) were used to transfect CV-1 cells, together with the indicated c- or v-erb-A-expression plasmids (or pKCR2, to keep the total amount of DNA used to transfect the cells constant). CAT assays were performed as described in Fig. 1*a*. Top panel: pMLV, no co-transfected pKCR2-CEA; bottom panel, p Δ MLV, no co-transfected pKCR2-CEA. *b*, Plasmid pMLV, in the presence of 0.2 μg pKCR2-CEA.

almost completely restored by co-transfection of a constant amount of the *c-erb-A*-expression plasmid pKCR2-CEA (Fig. 3b), again indicating that the *c*- and *v-erb-A* function in an antagonistic manner. Remarkably, under these conditions even a 25-fold excess of co-transfected *v-erb-A*-expression plasmid pKCR2-VEA (containing the *v-erb-A*-coding sequence in the same context as *c-erb-A*) only marginally decreased the T_3 response exerted by the combined endogenous and transfected T_3 receptor.

The inefficient competition of *v-erb-A* against *c-erb-A* activity prompted us to compare the relative affinities of both proteins for TREs. Figure 4 shows a gel-retardation experiment monitoring complex formation between the *c*-, *v*- and two *c/v*-chimaeric *erb-A* proteins, and two TRE-containing labelled DNA fragments derived either from an intron of the *RGH* gene¹⁷ (panel *a*) or from the MoMLV LTR (panel *b*). The chimaeric proteins are gag-*erb-A* hybrid proteins that bind T_3 with the same affinity as *c-erb-A* itself⁸. The V2 protein has four amino-acid substitutions, of which two are located between the gag- and DNA-binding domains and two in the DNA-binding region itself (see Fig. 4c). V3 has gag fused to *c-erb-A* as in *v-erb-A*, but has no other changes compared with *c-erb-A*⁸. The *erb-A* proteins were produced by vaccinia-virus expression, and equal amounts of *erb-A* products were used in the assay as determined both by SDS-gel electrophoresis and [¹²⁵I] T_3 binding (not *v-erb-A*) (data not shown). The results shown in Fig. 4a and b demonstrate that significant complex formation with both fragments occurred in the presence of *c-erb-A* (lanes labelled 3) and V3 (lanes labelled 6) containing extracts, but not with control vaccinia

extracts. The V3-DNA complexes migrated more slowly than the *c-erb-A* complex irrespective of the amount of V3 protein used (lanes labelled 6-9), presumably because of the higher relative molecular mass of the V3 protein. By contrast, the *v-erb-A* (lanes labelled 4) and V2 (lanes labelled 5) proteins displayed markedly reduced affinities for both fragments compared with the *c-erb-A* and V3. Complex formation with the MoMLV LTR fragment is obscured under these conditions because the extracts contain many other interacting proteins. In additional experiments, anti-*erb-A* antibodies co-precipitated TRE-containing DNA fragments of the growth-hormone gene complexed with the *c-erb-A* or V3, but not *v-erb-A* or V2 (data not shown). These results suggest that one or several of the four amino-acid substitutions in the *v-erb-A* and V2 proteins, of which two are in the DNA-binding region, lead to a reduction of their affinities for certain TREs. We do not know whether this reflects a generally reduced affinity of DNA-binding by *v-erb-A*, or a difference in specificity for particular *cis*-acting sequences, because no direct targets for *v-erb-A* are presently known. A residue corresponding to one of the three residues (207 in the human oestrogen receptor) responsible for the differences in DNA-binding specificity between the glucocorticoid and oestrogen receptors¹⁹ is also mutated in *v-erb-A* and V2 (Gly 73 in *c-erb-A* to Ser in *v-erb-A*). The V2 and V3 oncoproteins also have different effects on erythroid cell growth and differentiation (H. Beug, personal communication).

The data show that overexpression of *v-erb-A* in the presence of T_3 -receptor levels normally existing in cells, interferes with induction of transcription by thyroid hormone. It has also been proposed that repression of transcription has a role in the transforming potential of the adenoviral E1a protein²⁰, possibly through complex formation with, and functional inactivation of, other nuclear proteins (such as the product of the *Rb* locus)²¹. On the basis of our results, we propose that the dominantly acting *v-erb-A* oncogene exerts its transforming effects by causing the functional loss of normal (*c-erb-A*-mediated) cellular regulatory pathways, the result being operationally similar to the inactivation of a recessive proto-oncogene. Naturally occurring splice-variants of the cellular T_3 -receptor have also been proposed to act as general repressors of thyroid-hormone action in particular tissues²². Overexpression of *v-erb-A* by the avian erythroblastosis virus, however, and the changed DNA-binding behaviour of the protein as compared with the thyroid-hormone receptor raises in addition the possibility of changes in the target-gene specificity of *v-erb-A*. □

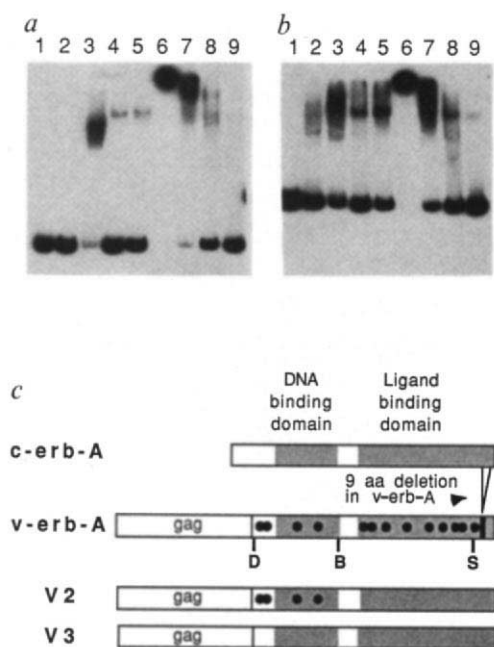


FIG. 4 Complex formation between *erb-A*- α proteins and TRE-containing fragments. *a*, An intron fragment from a rat growth-hormone gene containing a TRE (manuscript in preparation); *b*, MoMLV LTR fragment (described in Fig. 2) assayed by gel-retardation analysis. The probe (1 fmol) was incubated with 0.5–1 μ g nuclear extract as described in Fig. 2a, and the complexes formed analysed on non-denaturing, 6% acrylamide Tns-borate gels run at 4 °C. The amounts of extracts used were normalized by estimating the amount of *erb-A* protein using T_3 -binding assays and SDS gels stained with Coomassie Blue. Lanes labelled 1: no extract; 2: wild-type extract; 3: *c-erb-A*; 4: *v-erb-A*; 5: V2; 6: V3; lanes labelled 7–9: 2, 4 and 8-fold decrease in amounts of V3 extract used, respectively. *c*, Schematic representation of the various *erb-A* proteins. Dots indicate point mutations in the *v-erb-A* protein. D, B and S indicate restriction sites used for the construction of the chimaeric proteins. (For details see ref. 8.)

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A novel genetic system to detect protein-protein interactions

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PROTEIN-protein interactions between two proteins have generally been studied using biochemical techniques such as crosslinking, co-immunoprecipitation and co-fractionation by chromatography. We have generated a novel genetic system to study these interactions by taking advantage of the properties of the GAL4 protein of the yeast *Saccharomyces cerevisiae*. This protein is a transcriptional activator required for the expression of genes encoding enzymes of galactose utilization¹. It consists of two separable and functionally essential domains: an N-terminal domain which binds to specific DNA sequences (UAS_G); and a C-terminal domain containing acidic regions, which is necessary to activate transcription^{2,3}. We have generated a system of two hybrid proteins containing parts of GAL4: the GAL4 DNA-binding domain fused to a protein 'X' and a GAL4 activating region fused to a protein 'Y'. If X and Y can form a protein-protein complex and reconstitute proximity of the GAL4 domains, transcription of a gene regulated by UAS_G occurs. We have tested this system using two yeast proteins that are known to interact—SNF1 and SNF4. High transcriptional activity is obtained only when both hybrids are present in a cell. This system may be applicable as a general method to identify proteins that interact with a known protein by the use of a simple galactose selection.

The basic strategy of these experiments is shown in Fig. 1. The native GAL4 protein, containing both domains, is a potent activator of transcription when yeast are grown on galactose media. The N-terminal domain binds to DNA in a sequence-specific manner but fails to activate transcription². The C-terminal domain contains the activating regions but cannot activate transcription because it fails to localize to UAS_G (see, for example, ref. 4). Creation of two hybrid proteins, each containing one of the interacting proteins X and Y, allows the activating region of GAL4 to be brought to its normal site of action. Our test case for two interacting proteins uses the yeast SNF1 protein, a serine-threonine-specific protein kinase⁵ and the SNF4 protein, which is physically associated with SNF1 and is required for its maximal activity (J. Celenza & M. Carlson, personal communication).

The constructs used here are shown in Fig. 2: GAL4(1-881) is the entire GAL4 protein of 881 amino acids⁶; GAL4(1-147) is the N-terminal 147 amino acids of GAL4, which binds to UAS_G; GAL4(1-147)-SNF1 is a hybrid protein containing the N-terminal domain of GAL4 fused in-frame to the entire coding sequence (633 amino acids)⁵ of the SNF1 protein. The GAL4

N-terminal domain contains a nuclear-localization sequence⁷, so we expect that at least some fraction of the GAL4(1-147)-SNF1 would be localized to the nucleus. SNF4 is the entire SNF4 protein (322 amino acids; J. Celenza, F. Eng and M. Carlson, personal communication); SNF4-GAL4(768-881) is a hybrid protein containing all but the last amino acid of SNF4 fused in-frame to the GAL4 activating region II. This activating region is sufficient when fused to the GAL4 DNA-binding domain to induce substantial transcriptional activity³.

We introduced plasmids containing these different derivatives into strain GGY1::171⁸, which is deleted for both GAL4 and GAL80 (a negative regulator of GAL4) and which contains a *GAL1-lacZ* fusion gene integrated at the *URA3* locus. Thus β -galactosidase activity is a measure of GAL4 function derived from the plasmid-borne GAL4 constructs. The strain also contains mutations of the *HIS3* and *LEU2* genes, which are the markers for plasmids containing the DNA-binding and activation domains, respectively. Transformants were grown in media which can induce transcription from UAS_G (2% galactose, 2% ethanol, 2% glycerol) and which did not contain leucine or histidine, as appropriate to maintain the plasmids.

Our results are shown in Table 1. There was no activity in the absence of any GAL4 plasmid (line 1); 4,000 units of β -galactosidase were produced when the native GAL4 protein was provided (line 2). As expected, the DNA-binding domain alone did not activate transcription (line 3), nor did the GAL4(1-147)-SNF1 fusion protein (line 4), indicating that the SNF1 domain does not function as an activating region. The intact SNF4 protein (line 5) and SNF4-GAL4(768-881) fusion protein also failed to activate transcription. When both GAL4(1-147)-SNF1 and SNF4-GAL4(768-881) were introduced however, 180 units of β -galactosidase were produced (line 7), an induction

TABLE 1 Transcriptional activation produced by hybrid GAL4 proteins

Plasmid	β -Galactosidase activity
1. None	<1
2. GAL4(1-881)	4,000
3. GAL4(1-147)	<1
4. GAL4(1-147)-SNF1	<1
5. SNF4	<1
6. SNF4-GAL4(768-881)	<1
7. GAL4(1-147)-SNF1; SNF4-GAL4(768-881)	180
8. GAL4(1-147)-SNF1; SNF4	7
9. GAL4(1-147); SNF4-GAL4(768-881)	<1

Plasmids were introduced¹² into yeast strain GGY1::171 (ref. 8) deleted for GAL4 and containing a *GAL1-lacZ* fusion gene in the chromosome. Transformants were grown in 2% galactose, 2% ethanol and 2% glycerol and assayed in triplicate for β -galactosidase activity, as described^{4,9}.

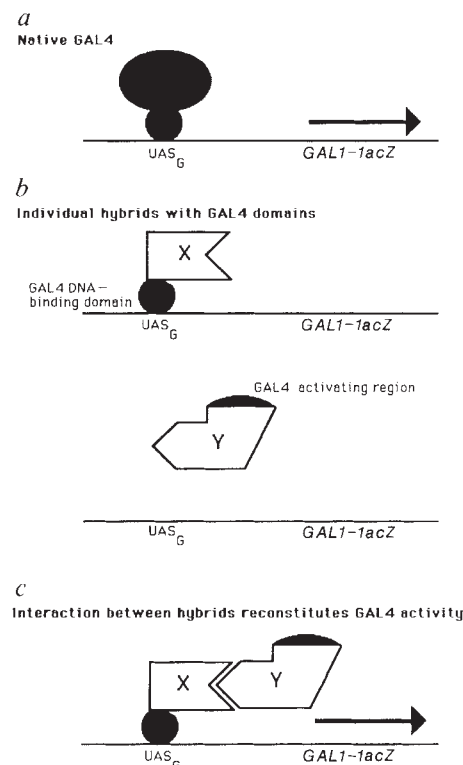


FIG. 1 Model of transcriptional activation by reconstitution of GAL4 activity. *a*, The native GAL4 protein contains both DNA-binding and activating regions and induces *GAL1-lacZ* transcription. *b*, Hybrids containing either the DNA-binding domain (upper) or activating region (lower) are incapable of inducing transcription. *c*, A protein-protein interaction between proteins X and Y brings the GAL4 domains into close proximity and results in transcriptional activity.

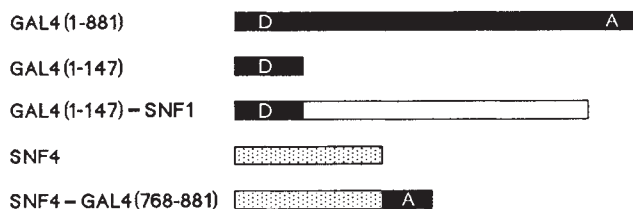


FIG. 2 Constructions used in our experiments. GAL4(1-881) is carried on plasmid pCL1, GAL4(1-147) on pMA424 (ref. 14), GAL4(1-147)-SNF1 on pEE5, SNF4 on pFF1 and SNF4-GAL4(768-881) on pNI12. D and A represent the DNA-binding and activating regions, respectively, of GAL4 protein.

METHODS. We constructed pCL1 by inserting the *Bam*HI fragment of pLKC15 (refs 3, 7) which contains the *P_{ADH1}-GAL4* gene into a YCP50 (described in ref. 15) derivative in which the *LEU2* gene replaces the *URA3* gene (from E. Stone). pEE5 is derived from pMA424 and pCC107 (from J. Celenza), which contains the *SNF1* gene in pUC18 (ref. 16) as follows. A *Pst*I site 12 base pairs upstream of the initiator methionine of *SNF1* was converted to a *Bam*HI site and then the *Bam*HI fragment containing the *SNF1* gene was inserted into the *Bam*HI site of pMA424; pFF1 was constructed by inserting the *Hind*III fragment of pFE27-2 (from J. Celenza, F. Eng and M. Carlson), which contains the *SNF4* gene, into the multi-copy yeast plasmid YEp13 (ref. 17). pNI12 was constructed from a pUC18 clone containing the *GAL4* gene and *ADH1* terminator (derived from pLKC15) from the *Nar*I site at residue 767 of *GAL4* to the 3' *Bam*HI site. The *Nar*I site was converted to a *Kpn*I site, and the resulting *Kpn*I fragment ligated to pSL321 (from J. Celenza) at a *Kpn*I site 6 base pairs downstream of the penultimate amino acid of the *SNF4* gene. Finally, a *Hind*III fragment containing the *SNF4* promoter and gene fused to the GAL4(768-881) region was inserted into YEp13.

of several hundredfold over the background level of the single hybrids. Although the activity produced by the two hybrids is only 4.5% of that produced by the native GAL4 protein expressed from the strong *ADH1* promoter, it is sufficient to produce a dark blue colony on plates containing the indicator X-gal. Thus yeast cells containing the interacting hybrid proteins can be detected easily against a background of cells which contain only a single hybrid and are white.

This induction with the two hybrids does not result from the SNF4 protein binding to the GAL4(1-147)-SNF1 hybrid and converting it to a transcriptional activator; introduction of a plasmid encoding the intact SNF4 protein into cells carrying this hybrid was not sufficient for such high activity (line 8), although a low level of activity was observed. Furthermore, the induction is not due to the GAL4 activating region interacting with the GAL4 DNA-binding region without the need for the SNF1-SNF4 interaction (line 9). The transcription produced by the combination of the GAL4(1-147)-SNF1 and SNF4-GAL4(768-881) hybrids is strictly dependent on inducing media, as this and all other combinations of hybrid proteins produced <1 unit of β -galactosidase activity in glucose media.

Our results extend those of a previous study⁹ which indicate that the GAL80 protein can be converted into a transcriptional activator by the insertion of an acidic sequence and allowing the resultant protein to interact with a DNA-bound GAL4 derivative. Similarly, analysis of the herpes simplex virus protein VP16 indicates that it can activate transcription by binding to host proteins bound to DNA¹⁰ and analysis of the protein product of *c-fos* indicates that it can activate transcription by binding to the DNA-bound *c-jun* protein product¹¹. These examples, however, involve proteins naturally involved in regulating transcription, whereas the system we describe should be applicable to almost any two interacting proteins. The system requires that the interaction can occur within the yeast nucleus, that the GAL4-activating region is accessible to the transcription machinery, and that the GAL4(1-147)-protein X hybrid is itself not a potent activator.

We envisage that this system could be used as a method for the genetic selection of proteins that interact with a known

protein, if the cloned gene for the known protein is available. Yeast containing this protein as a GAL4(1-147) hybrid would be transformed with a clone bank of genomic or complementary DNA sequences fused to the GAL4 activating region. The double transformants would be selected for ability to grow on galactose or screened for blue colour on indicator plates for those able to express the *GAL1-lacZ* fusion. Such an approach could be useful in identifying, for example, proteins that interact with oncogene-encoded products, or for mapping the specific interacting domains of proteins known to form a complex. Additionally, it could be of value in designing therapeutic peptides to interact with proteins of bacterial or viral origin. □

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CORRIGENDUM

An eIF-4A-like protein is a suppressor of an *Escherichia coli* mutant defective in 50S ribosomal subunit assembly

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IN this letter parts of the DNA sequence and thus the derived protein sequence are revised at the following positions: nucleotides from position 610 to 615 are -ggcacc- encoding the amino acids GT. The nucleotides g (713), c (850) and g (874) are deleted. The amino acid sequence corresponding to the new reading frame from nucleotides 712 to 873 is TRELAMQVSDHARELAKHHTLDIATITGGVAYMNHAEVFSNQDIVVATTGRLL. The sequence from position 1233 to 1246 is -gctgagcgc-. One -g- is inserted between nucleotides 1277-1278 and between 1280-1281. The corresponding new amino acid sequence from nucleotides 1231 to 1281 of the original sequence is KRERVHELANWLREAG. Identical amino acids between eIF-4A and the SrmB protein increase to 108 out of 382 amino acids (28.3%).

Since the uncorrected protein sequence of the *srmB* gene was contributed by K. Nishi and J. Schnier to the scientific correspondence by P. Linder *et al.* (*Nature* **337**, 121-122; 1989), the new sequence also changes the number of identical amino acids among the eIF-4A-like proteins described in that publication. The amino acids which now are common to all 8 eIF-4A-like proteins increase to 48/408 (11.8%). The SrmB protein shows divergence from this set of proteins at only 4 positions. □